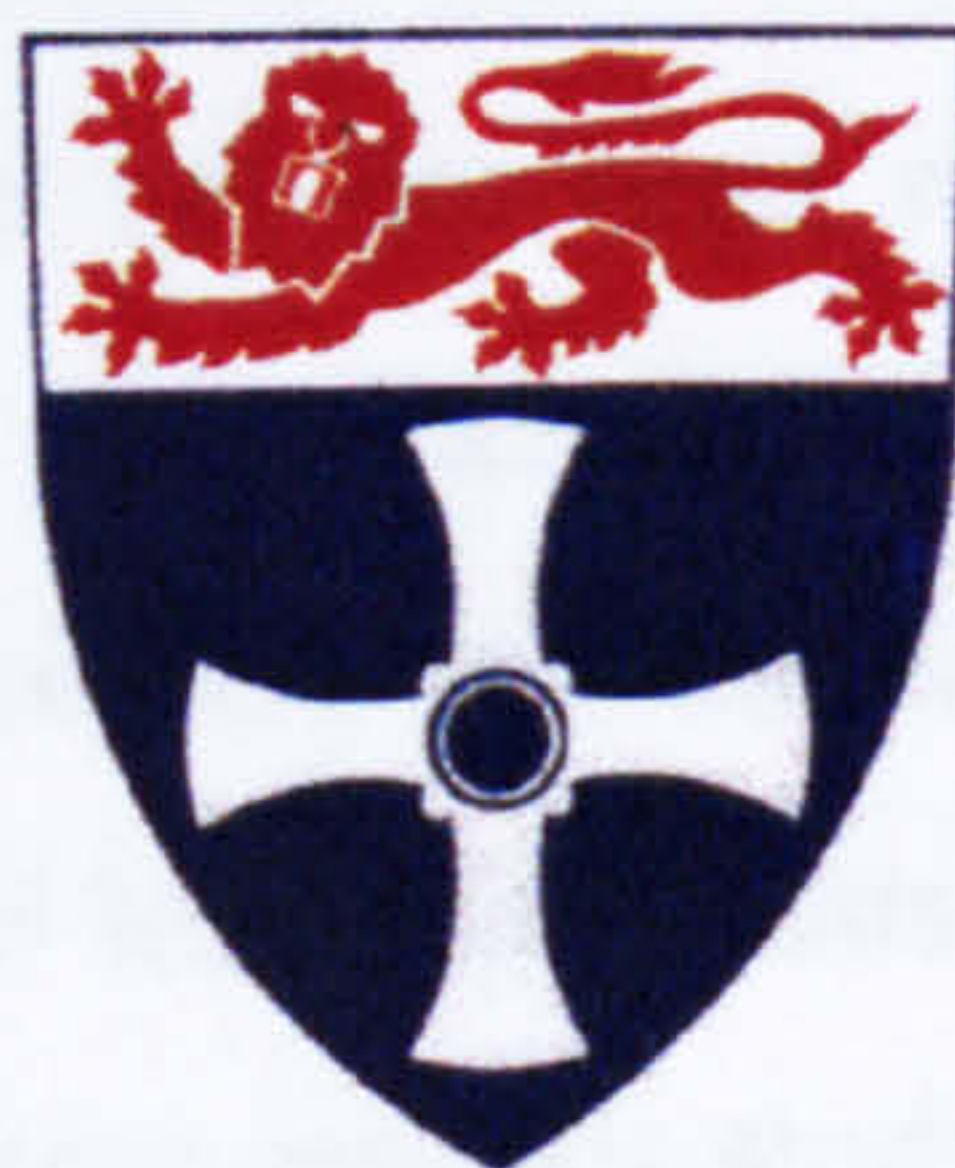


INVESTIGATION INTO REMEDIATION OF CONTAMINATED SOIL CONTAINING HIGH SULPHATE AND HEAVY METALS CONCENTRATION



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ABSTRACT

This study involved the investigation of a contaminated soil problem in Gateshead, UK. The site was previously a dumping area from industrial activities for over a hundred years and generated problems of high sulphate concentration and heavy metals in both the soil and the leachate which discharges into the River Tyne. The combination of such contaminants has not been widely investigated in the area of contaminated soil. The study was therefore divided into 2 parts, namely bioremediation of the contaminated soil and leachate treatment by reverse osmosis. The bioremediation study involved treatability tests which included slurry, microbial growth and column tests. The reverse osmosis study involved membrane fouling and leachate pre-treatment experiments.

The bioremediation study stimulated the indigenous microorganisms by the addition of nutrients and carbon sources. The soil slurry and microbial growth tests determined the combination of nitrogen and phosphorus required to produce higher CO₂ evolution as an assessment of microbial activity. It was found in the column tests that the addition of a carbon source and appropriate nutrient combinations resulted in a significant reduction of sulphate in both the leachate and the soil matrix. Furthermore, this was also accompanied by an increase in the microbial population in the soil matrix. It was also considered that assimilatory sulphate reduction by microorganisms had taken place since H₂S production could not be detected in the open system of the column. However, the high pH of the soil that was higher than 8 possibly caused H₂S production undetected in this study.

Zinc, manganese and copper, in contrast were not reduced in the soil matrix. Only arsenic showed significant reduction in the soil columns. Heavy metals were precipitated and were still present in high concentrations in the leachate and would require further treatment in the liquid phase. This was demonstrated by the study of the use of a LPRM (Low Pressure Reverse Osmosis Membrane) to treat leachate from the contaminated soil.

The reverse osmosis study showed that zinc and arsenic could be reduced by up to 86% and 97% respectively. Sulphate was also satisfactorily reduced up to 99%. However, the study on membrane fouling confirmed that the sulphate concentration was the main effect of fouling.

Ferric chloride, aluminium sulphate, barium chloride and polyelectrolyte Zetag 92 were used for coagulation-flocculation in the pretreatment of the leachate. The study revealed that the sulphate concentration could only be reduced at the most by 43% using a FeCl_3 , BaCl_2 and Zetag 92 combination. FeCl_3 showed better floc characteristics than alum whereas BaCl_2 improved sulphate removal but increased the turbidity in the supernatants. However, the use of BaCl_2 would significantly increase the cost of pretreatment.

The study recommended a further investigation into the use of a range of readily available carbon, nitrogen and phosphorous sources in the soil column or at pilot-scale for designing a full-scale bioremediation system. Meanwhile, an investigation into other leachate pretreatment methods such as continuous microfiltration or anti-scalant addition was also suggested.

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LIST OF ABBREVIATIONS AND SYMBOLS

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
APS	Adenosine-sulphato-phosphate
ATP	Adenosine triphosphates
C	Carbon
CA	Cellulose acetate
CARACAS	Concerted Action on Risk Assessment for Contaminated Sites
CEST	Centre of Exploitation of Science and Technology
CCMS	Committee on the Challenges of Modern Society
CFU	Colony forming unit
COD	Chemical oxygen demand
DF	Degree of freedom
DoE	Department of Environment
DTI	Department of Trade and Industry
EDTA	Ethylene diamine tetra acetic acid
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
GC	Gas chromatography
GMBC	Gateshead Metropolitan Borough Council
HPC	Heterotrophic plate count
ICRCL	Interdepartmental Committee on Reclamation of Contaminated Land
kPa	Kilo Pascal
LOI	Loss on ignition
LPROM	Low pressure reverse osmosis membrane
MPa	Mega Pascal
MLVSS	Mixed liquor volatile suspended solids
mM	millimolar
N	Nitrogen
NADH	Nicotinamide adenine dinucleotide phosphate
NAPL	Non-aqueous phase liquid
NICOLE	Network of Industrially Contaminated Land in Europe
O	Oxygen
P	Phosphor
PAPS	Adenosine 3'-phosphate 5'sulphate-phosphate
PAH	Polynuclear aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PPi	Pyrophosphate inorganic
RBC	Rotating biological contactor
RO	Reverse osmosis
S	Sulphur
SBR	Sequencing batch reactors
SDI	Silt density index
SHMP	Sodium hexa metaphosphates
SRB	Sulphate reducing bacteria

SPP	Sodium pyrophosphate
STD	Standard deviation
TDS	Total dissolved solids
TFC	Thin film composite
TOC	Total organic carbon
UAF	Upflow anaerobic filter
VSS	Volatile suspended solids
VFA	Volatile fatty acids
WHC	Water holding capacity
D	Distance
F	Force
F _o	Ratio of means squares of treatment to means squares of error
G ⁻	Gram negative
ΔG	Free Gibb's energy
K _{ow}	Octanol-water partition coefficient
MS	Means squares
pE	Potential electron
pKa	Logaritmic of solution equilibrium
SS	Sum of squares

CHAPTER 1

INTRODUCTION

Land is unique and possesses complex properties since it is the interface between the lithosphere, atmosphere, and biosphere. On the other hand, land very often is a sink for many kinds of wastes in the earth. Sludge, solid waste, and some hazardous wastes have been disposed of, or contained in the land or the subsurface. Even air pollution has resulted in acid rain precipitation which can affect its quality. Therefore, land contamination may be present in many sites as a result of waste disposal or from previous industrial use.

Increased awareness and concerns about environmental issues have now made land one of the important pollution sources in society. The issues has emerged over the last 20 years in the UK, as a result of a number of well publicised incidents in the 1980s and 1990s. Identification and quantification of any land contamination are becoming substantial as a result of growing interests in environment liability and increasing development pressure.

When is a land actually classified as contaminated? There are some definitions concerning contaminated land. The Environmental Protection Act 1990 under section 78A(2) provides guidance on the definition of contaminated land as:

"any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land, that-

- a). significant harm is being caused or there is a significant possibility of such harm being caused; or
- b). pollution of controlled waters is being, or is likely to be caused."

The Interdepartmental Committee on Reclamation of Contaminated Land (ICRCL) of the Department of Environment (DoE) set up guideline on contaminated land in Notes 59/1983 which listed concentration limits on pollutants to determine any action for a particular site.

In 1993, the amount of contaminated land in the UK was estimated to be between 100,000 to 220,000ha which represents 0.4% to 0.8% of total UK land area (Pollard and Herbert, 1998). This did not differ from the estimation of the Centre for the Exploitation of Science and Technology (CEST) who suggested that the potential contaminated land area in the UK to be in the range of 50,000 - 250,000ha. The number of sites has been estimated as 100,000 (Bardos, 1994).

Treatment approaches have also changed because of recent domestic and EC environmental legislation and the emergence of new technologies. Policies on contaminated land in the UK are given in Section 57 of the Environmental Act 1995 as: (Pollard and Herbert, 1998)

- i). maintenance of the 'suitable for use' approach,
- ii). dealing with urgent and real problems in an orderly and controlled fashion,
- iii). the creation of greater clarity and certainty than the law currently provides, and
- iv). replacement of the existing statutory nuisance powers with a modern, specific contaminated land power, with extension to Scotland of the statutory nuisance provisions already existing in England and Wales.

Although there have been many methods and techniques studied for remediating a contaminated soil, the problem can only be approached site specifically. There are few, if any, identical conditions found at contaminated land sites since the physical, chemical and microbiological components will differ between one site and another. These together affect the appropriateness or suitability of methods applied to that particular site. Solving contaminated soil problems usually requires interdisciplinary experts. Acknowledging this requirement therefore, this study may only concentrate on the technical considerations of applying a particular remediation method.

The study carried out was to investigate remediation methods that could be applied to a specific contaminated land area in Gateshead, UK. The area used to be a waste dumping area from over a hundred years ago. The Gateshead Metropolitan Borough Council (GMBC) has provided some evidence that the site has resulted in environmental problems to the neighbouring area. Sulphide gas can be detected in an industrial storage building close to the site and also at the surface of the site. Some

leachate discharge to the River Tyne have also been monitored and shown to contain high sulphate levels and which has raised concern on its effects on the fish life in the river.

Due to the source of the problem in the Gateshead contaminated area, the remediation approach was divided into 2 main phases. Soil, or ground contamination was proposed to be treated by a bioremediation method whereas the leachate was considered to be best treated by a reverse osmosis application. The objective of both bioremediation and reverse osmosis applications were to reduce the sulphate and some heavy metals such as zinc, manganese and copper contents from the contaminated soil and leachate studied.

Although the study was undertaken on samples from the area it could not cover the whole area which spreads over 25 hectares. Therefore, the study was aimed at providing a greater understanding of the processes and problems faced when applying those remediation methods selected.

CHAPTER 2

LITERATURE REVIEW

2.1 Problems Caused by Contaminated Land

Soil contamination can originate from industrial activities, waste handling facilities and landfill, agricultural activities and any organization which broadly uses chemicals. Not only are industries that manufacture chemicals the source of contamination, but industries that use the chemicals may generate a potential by-product which could be considered hazardous. In 1987, the US-EPA collected 74,000 reports on toxic hazardous waste chemicals from 19,000 industrial facilities and from which 50% were from manufacturers producing chemicals, with the other 50% being from businesses that used chemicals (Cookson, 1995). Identification of specific chemicals which are hazardous becomes important to determine their toxicity to the environment

The US-EPA, in 1980, identified 31,000 abandoned waste sites. This did not include those which were not in the EPA priority list. From this number, there were an estimated 19,000 sites which required clean-up in addition to 295,000 leaking underground storage tanks. It was also noted that a significant percentage of hazardous spills were petroleum based, volatile solvent based or polynuclear aromatic hydrocarbons (PAHs). A small percentage included pesticides, polychlorinated biphenyls (PCBs) and others (Cookson, 1995). In Germany, there were 190,716 suspected sites recorded, 87,444 of which were abandoned waste disposal sites and 107,272 were abandoned industrial sites (Sanden & Freier, 1998).

According to an EPA report (Cookson, 1995), the most prevalent contamination of soil and groundwater was by petroleum hydrocarbons due to the widespread use and storage of petroleum fuels. The source of contaminants included chemical storage tanks, oil-water separators, refining facilities, crude oil and fuel storage, drilling mud, oil field brine, and service stations. The petroleum mixtures and sludges may vary according to their origin, storage, treatment and weathering condition. Petroleum contaminants frequently contain a large mixture of hydrocarbons, e.g. jet fuel contains

over 300 different hydrocarbons.

The second most common contaminants were creosotes which is a mixture of over 200 major chemicals. Creosote contains approximately 85% polynuclear aromatic compounds (PAH), 12% phenolic compound, and 3% heterocyclic compounds.

The third most common contaminants were volatile organic compounds, of which one portion of the volatile chemicals originate from hydrocarbon spills. These are the aromatic volatile fraction of benzene, toluene, ethyl benzene and xylene (BTEX compounds). Other volatile organics that often found are halogenated solvents that are used as solvents, cleaning solutions and chemicals synthesis.

More recently concern over soil contamination has expanded to inorganic contaminants such as heavy metals. Their existence with some organic wastes or volatile organic compound products have been reported in many contaminated soil situations. Under US-Department of Energy sites and military bases, the most prevalent metals in groundwaters were Pb, Cr, As, and Zn with the major anions being NO_3^- . In soils and sediments the most frequent inorganic compounds were Cu, Cr, Zn, Hg, As, Cd, and NO_3^- (Sparks, 1995).

Radionuclides or radioactive wastes have also been found in some industrial wastes under collaborative research by the NATO/CCMS Pilot Study Programme. Kvasnikova et al. (1998) studied the vertical migration of radionuclides in the soil after the Chernobyl accident. The most common radionuclides found in the groundwater were tritium, U, and Sr whereas in soils or sediments the most common were U, Pu, and Cs (Spark, 1995).

The types of contaminants which were considered in the NATO/CCMS Pilot Study were also reflected similar situation. Out of 52 projects, 40 dealt with organic contaminants such as PAHs, PCBs, and BTEX compounds, 6 involved metals and similar numbers tackled mixtures of inorganic and organic, whereas only one project was concerned with sulphate and cyanide (Smith et al., 1998).

In the UK, site redevelopment not only considered with hazardous wastes from the chemicals industries but also from derelict land or abandoned building or industrial facilities. Problems from hazardous gases such as methane were often found in landfill areas (Braithwaite, 1994).

2.2 Soil Remediation Techniques

2.2.1 Types of technologies

Treatment technologies for contaminated soil or groundwater can be divided into categories which depend on their general operating principles, namely biological, chemical, physical, solidification or thermal. According to the location of the treatment process, remediation can be carried out *in situ* (applied at the contamination site) or *ex-situ* (involving removal of the contaminated soil by excavation and then treatment on site or transported to another location). *In situ* treatment reduces exposure risks because the contaminated soil does not need to be excavated and transported. On the other hand, *ex situ* alternative may give rise to hazards to the workers or to the surrounding area during transportation.

Remedial process categories can be described as follows (Bardos, 1994):

- i). Biological processes which depend on the biological transformation or mineralization of contaminants to less toxic, more mobile forms or a form which is less toxic and mobile.
- ii). Chemical processes destroy, fix or neutralize toxic compounds and is important in solidification/stabilization processes.
- iii). Physical processes remove contaminants from the soil matrix or groundwater. The concentrates will then require further treatment or disposal. They may be destroyed or recovered by other process before disposal.
- iv). Solidification processes encapsulate contaminated substances in a monolithic solid of high structural integrity. Stabilization is a solidification process which is also accompanied by chemical fixation. Vitrification is a form of solidification using high temperatures to fuse contaminated matter.
- v). Thermal processes include incineration, gasification, desorption, volatilization,

pyrolysis, or a combination of some of these processes.

Some soil treatment technologies, which are often used to treat specific contaminants are as follows:

i. *In situ* treatments

Volatilization

Volatilization is used to recover volatile contaminants such as volatile organic carbon materials. *In situ* volatilization consists of mechanical drawing or air venting which causes air to flow through the soil via a slotted or screened pipe. The volatile matter will then be released with the air and the decontaminant soil particles remain.

Other methods which may be classified as volatilization include vapour extraction by vacuum, steam or hot air injection. Important parameters are vapour pressure of the contaminants, soil permeability, porosity, particle size distribution, depth of contaminants and the water table level (Sims, 1990).

Biodegradation

This involves the enhancement of naturally occurring microorganisms by stimulating their number and activity. The soil contaminants are then degraded by the microorganisms. Factors affecting the effectivity of biodegradation include moisture content, pH, temperature, the initial microbial community, and the availability of nutrients. It is important to realize that microbes cannot effectively degrade all pollutants. A microbe may effectively reduce some contaminants but not others. This method is therefore applicable to contaminants which are biodegradable and can be aerobic, or anaerobic or both.

Leaching

Leaching involves leaching the *in situ* soil with water or often with surfactants to remove contaminants. A surfactant is a surface active agent that contains hydrophilic and hydrophobic regions to lower the surface tension. The leachate from the soil is collected for disposal or further treatment. The effectiveness of leaching depends on

permeability, homogeneity, soil texture and mineralogy all of which affect the release of the contaminants from the soil and the leaching rate of the contaminants through the soil. The method however has limitations since it requires a large quantity of water and also higher costs for the waste stream disposal. The method is also referred to as soil flushing.

Vitrification

In situ vitrification consists of immobilizing the contaminant by solidification with an electric current. It can immobilise contaminants for in excess of 10,000 years. The cost is high due to the high energy requirement.

Isolation/containment

Contaminants may be contained in place by installing subsurface barriers such as clay liners and slurry walls to minimise the migration of contaminants.

Passive remediation

This method allows natural processes such as volatilization, aeration, biodegradation and photolysis to occur. It is an inexpensive remediation method that requires careful site monitoring for any contamination. Factors affecting passive remediation include biodegradation, adsorption, volatilization, leaching, photolysis, soil permeability, groundwater depth, infiltration and the nature of the contaminants.

ii. *Ex situ* treatments

Land treatment

In this process the contaminants are excavated and spread over an area of land so that natural processes can occur in order to remove the contaminants. Nutrients are supplied to stimulate the microorganisms. The soil can be mixed with other soils to enhance the contact area of the microorganisms and contaminants and to increase aerobic condition.

The addition of compost may be applied to enhance the natural process, however, a large quantity of compost is usually required.

Thermal treatment

In thermal treatment the excavated soil is exposed to heat or steam in order to break down the pollutants and to release the volatile matter which is then collected and moved through an afterburner and recovered with solvents.

Asphalt incorporation

Contaminated soil could be mixed with hot asphalt and the mixture used in paving. Heating the mixture causes volatilization or decontamination of some of the contaminants and paving will contain the remaining contaminants.

Solidification/stabilization

Here, contaminated soil is encapsulated by the addition of additives to excavated soil and landfilling the mixtures. The contaminants are not destroyed and therefore cannot be moved. This method is employed to contain inorganic contaminants.

Chemical extraction

In this process the excavated soil is mixed with solvents or surfactants or a solvent/surfactant mixture to remove the contaminants. The released contaminants and the remaining solvent/surfactant are then separated from the soil which is washed or aerated to remove solvent/surfactants and is then filtered for fine particles. This method is expensive.

Excavation

Excavated contaminated soil may be removed and disposed of elsewhere in a landfill which is equipped with an impermeable liner to decrease the distribution of contaminants. The landfilled area should have a low soil permeability. This method requires a large area and possesses some disadvantages such as hazard to humans, safety control, odour production, and potential run off and groundwater contamination.

Biodegradation

Biological processes can also be employed for *ex-situ* treatment in slurry reactors above the surface (*on site*) or in a hazardous waste treatment plant (*off site*). The soil

treated must be amenable to biodegradation and a relatively small amount to ease transportation and permit safety handling.

2.2.2 Approach to remediation techniques

In 1980 the US-EPA enacted the first comprehensive federal law on releasing hazardous waste. It was called the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) or Superfund the goal of which was to establish mechanisms to respond to releases of hazardous substances from abandoned or uncontrolled hazardous waste sites which may threaten humans and the environment. Under CERCLA, the EPA will designate a hazardous waste site as a Superfund site and manage the clean up of the site. At Superfund sites, hazardous substances often contaminate soil, groundwater, or nearby streams or lakes. Superfund sites included landfills, abandoned mines, or industrial sites where hazardous materials were used or disposed.

In the UK, the DoE, under the auspices of the Environment Agency, carried out research on site remediation. There were 47 industries involved in the programme (Denner, 1997). The UK Department of Trade and Industry (DTI) promotes collaborative link with universities and industry into the biological treatment of soil and water. A budget through various sources, of £ 4.6 million was made available for areas including reclamation of polluted land (Bardos, 1994).

Increased awareness of the remediation of contaminated land was not confined to individual countries. Collaborative projects to disseminate and investigate problems of the applicability of techniques was initiated by NATO/CCMS in 1980. Their Pilot Study during 1992-1997 reviewed over 50 projects in 14 European countries. There were also many bilateral projects between two countries such as collaboration between Netherlands and the USA. In the period 1996-1998, the European Commission under the Environment and Climate Programme initiated CARACAS (Concerted Action on Risk Assessment for Contaminated Sites) was launched to coordinate current research initiatives on contaminated land risk assessment. This involved 16 European countries. Another institution funded by the European Commission DG XII and led by industry

was NICOLE (Network of Industrially Contaminated Land in Europe). This organization was introduced to exchange remediation experiences (Kasamas et al, 1998). They elaborated an information system concerning techniques and experiences in contaminated land problems which may give benefit to many other stakeholder of contaminated land issues.

2.2.3 Status of technology

Types of technology vary and develop according to the equipment and procedures used to apply the main processes. Many of these, called innovative technologies, are proposed and available to be evaluated for their effectiveness to treat certain contaminants. Countries such as the Netherlands and the USA conduct much research to classify the status of innovative remediation techniques. The Superfund programme in the USA classifies the status into three categories namely commercial, demonstrated, and emerging/experimental or possible. Commercial means the technique has been proven at field study or full scale, demonstrated implies close to commercial but may in some aspect lack full-scale experience. Emerging technology is proven successful in the laboratory and in pilot-scale experiment.

In the Netherlands the classification of technologies was divided into two categories, namely, intensive and extensive technologies. Intensive uses sophisticated technology and requires long initiation, running and support and thus makes it expensive to operate. This includes soil washing or thermal treatment. Extensive technology has low resource requirements which may take longer to become effective. This is relatively cheap and poses a smaller impact on soil. Examples of extensive processes are composting and using plants to remove metals.

In the UK, the DoE also carried out research into the contaminated land problem under the auspices of CIRIA. The DoE research programme involved 5 projects on evaluating innovative technology during 1993. Compared to some European countries and North America, the application of innovative technology in the UK has been relatively limited. In many cases, innovative treatment does not offer or is not believed to offer, a complete and cost effective solution to contaminated site problem (Bardos,

1994).

To enhance the effectiveness of soil treatment it can be carried out by process integration or also by a so called 'treatments-train' which combines two or more technologies consecutively or in series to treat a specific site problem. It may also be a mixed system that uses two or more techniques to treat a different contamination area or media across a site as part of an overall remedial strategy.

Under the NATO/CCMS Pilot Study on the Evaluation of Demonstrated and Emerging Technology for the Treatment and Clean up of Contaminated Land and Groundwater, the study involved 50% demonstrated and 50% emerging technologies. *In situ* treatment covered 18 projects, *ex situ* consisted of 26 projects, and 6 projects combined *in situ* and *ex situ*. According to the process categories, 24 projects were biological, 29 projects used physical and chemical processes, 4 projects implemented chemical treatment, 5 projects applied thermal process, and 2 projects used solidification/stabilization. Integration treatment systems were carried out in 19 projects, mixed systems were in 7 projects. Single treatment technologies were applied for 23 projects (Smith et al., 1998).

2.2.4 Treatment selection

Soil treatment technology can be selected according to many factors such as the nature of the contaminants in question, site investigation, funds available, regulations, perception of the community, etc. This may be a complex and difficult decision before carrying out further treatability test. Data bases and information exchange will then be very useful tools. Penmetsa and Grenney (1993) developed a computerised system called STEP (Soil Treatment Evaluation Program). However, the decision can only be made after a thorough study of site and chemical investigation.

2.3 Bioremediation

Bioremediation is the application of biological process principles to treat groundwater, soil, and sludge contaminated with hazardous chemicals (Cookson, 1995). Bioremediation is a practical solution to destroy completely or mineralization of

organic compounds without using incineration.

Compared to pump and treat methods for groundwater remediation, *in situ* bioremediation treats contaminants in place and may speed the clean-up process. This will usually reduce the total remediation cost. For soil treatment, *in situ* methods offer minimal disruption to the site. Other advantages of bioremediation include long-term protection of public health, positive public acceptance and can be coupled with other techniques. Bioremediation was considered to be more effective for treating PAHs *in situ* from a large manufactured gas plant (Cutright and Lee, 1994) and a reliable alternative for petroleum hydrocarbons (Autry and Ellis, 1992). Some disadvantages of bioremediation may also however, be considered. These include extensive monitoring needs, some chemicals cannot be bioremediated, site specific requirements, toxicity of contaminants, scientifically intensive, potential production of unknown by-products, and the perception of unproved technology. According to the Superfund remedial action data from 1982-1992, bioremediation were used in 9% of the total actions covering 5% surface bioremediation and 4% *in situ* bioremediation (Cookson, 1995).

A biological process is only reliable if the biochemistry and necessary environmental conditions are controlled and understood. The process is controlled to bring about a specific microbial transformation or degradation. It is more however, intricate because it uses catalysts (enzymes) from the microorganisms to catalyse the destruction of specific hazardous compounds. In general, the microbial catalysed reactions can be seen in Fig. 2.1

The hazardous compounds may or may not be substrates. The reaction of catalysed chemicals is conducted within the cell or outside the cell. The principal reactions are oxidation-reduction which are necessary for microorganisms for energy generation. Environmental control is required to produce the catalysts and desired reactions.

Many hazardous compounds which used to be considered as recalcitrant are degradable. This persistence is related to the environmental conditions which support microbial activity. There are two important concepts for bioremediation:

- i). the desired reactions, which may require a specific electron acceptor, and
- ii). any single microbial species with an ability to transform.

Organisms are highly specialised and play a very specific role in the mineralization of organic compounds. Thus, bioremediation requires containing, optimizing, and controlling nature's biological and chemical systems.

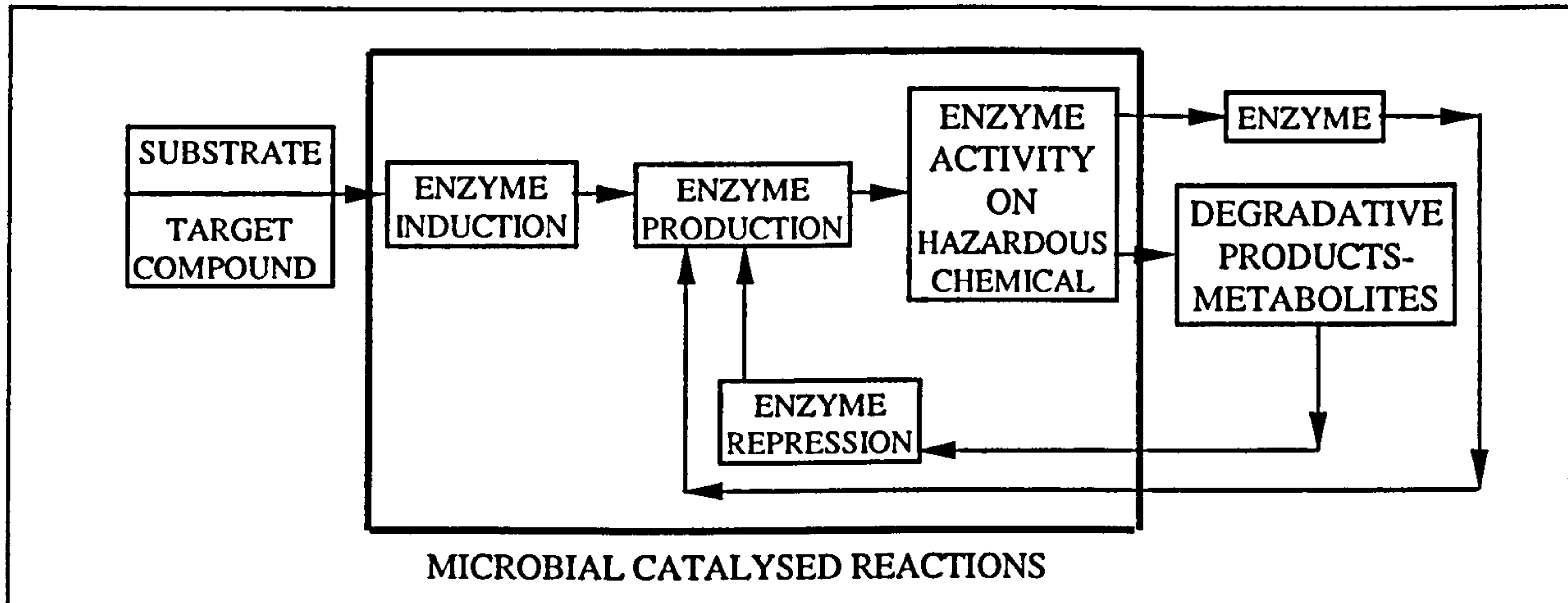


Figure 2.1. Generalised microbial catalysed reaction

Source: Cookson, 1995

Bioremediation can be successful if the process is controlled with the presence of a suitable energy source, an electron donor-acceptor system, and nutrients. These complex systems are frequently undertaken without a complete knowledge of their operation. Bioremediation therefore, frequently requires pilot-scale tests. The problems of scale-up of results or design from a bench- or pilot-scale study to a field situation may also be encountered.

2.3.1 Factors affecting bioremediation

i. Contaminants susceptible to bioremediation

Chemical and microbiological properties jointly affect the prospects for bioremediation. The key properties are the contaminants' tendency to sorb to subsurface solids and to partition into a non-aqueous phase that travel separately from the groundwater (Ritmann et al., 1994). The non-aqueous phase liquid (NAPL) may form if the contaminants are present in a high concentration. This may exclude water or air in the pores of the subsurface which restrict access to the remedial fluid or gases. Sorption and non-aqueous formation decrease the contaminants' availability to the

microorganisms. In lower concentrations, contaminants are essentially non-mobile and occupy less pore space than water. Rogers et al. (1993) considered unfavourable factors to bioremediation to include the complex mixture of contaminants, sparse microbial activity, absence of appropriate electron acceptors, and extremes of pH.

Bioremediation as a method of treatment has been established successfully to remediate petroleum hydrocarbon such as gasoline, fuel oil, alcohol, ketones, and ester. Ritmann, et al. (1994) summarised the status of the application of bioremediation for some other compounds:

- i). Ether bonds are found to be more resistant to microbial attacks.
- ii). Halogen molecules in organic compounds changes their solubility, volatility, density and toxicity. The presence of halogen molecules decreases susceptibility to microbial metabolisms. Dechlorination however, can be obtained in anaerobic microbial processes as shown in tetrachloroethene for aliphatic compounds.
- iii). Aromatics such as polychlorinated biphenyls (PCBs) are susceptible to aerobic and anaerobic processes.
- iv). Similarly, nitroaromatic compounds can be converted into CO_2 , H_2 or minerals by aerobic and anaerobic process.
- v). With regards to metals, microorganisms cannot destroy them, but may alter their reactivity and mobility. This has been widely used in mining industries. Microbes produce acids that can leach metals such as copper from low grade ores. Microbes also have the ability to demobilise metals by transforming them into precipitates.

ii. Environment affecting bioremediation

Bioremediation is not only affected by the contaminants' properties but also the site geological and chemical characteristics. A site is ideal for bioremediation if it is controllable and easy to interpret as in the laboratory flask experiments. This includes uniform geology and favourable chemical characteristics. This also includes granular porous media, and saturated conditions (Rogers et al., 1993). These conditions unfortunately are very rare. Soil type, geological strata, and water chemistry vary from site to site or even within an individual site. Therefore, more information is required

before implementing a bioremediation method. It is also possible that the degradation mechanisms for two occurring contaminants are mutually exclusive. In this case, a sequential treatment method may be needed.

2.3.2 Types of bioremediation

According to the definition applied in the USA, bioremediation can generally be classified into two categories namely intrinsic and engineered bioremediation (Rittmann et. al, 1994; MacDonald & Rittmann, 1993)

i. Intrinsic bioremediation

Intrinsic bioremediation depends on the capability of a naturally occurring microbial community to degrade a contaminant. There is no engineered step involved in enhancing the process. This, however, differs from no-action because it requires a thorough documentation of the role of native microorganisms to remediate the contaminants. This has to be performed at field sites, with on-site derived sample soil, sediment or water. The effectiveness of natural bioremediation can be determined by routine site monitoring. The method is also termed as natural, passive, spontaneous bioremediation and bioattenuation.

In intrinsic bioremediation, the site conditions should be accepted as constraints to the process. The critical property is the predictability of groundwater flow in time and space to determine whether:

- i). the indigenous microbes will be able to act in all places where contaminants may travel continuously through seasons, and
- ii). the microbes can act quickly enough to prevent any spreading of contaminants with groundwater flow.

To ensure these conditions, the water table should not fluctuate more than 1m through a seasons. Other site conditions which favour intrinsic bioremediation are:

- i). the presence of minerals such as carbonates in the aquifer which may buffer pH changes,
- ii). high concentration of oxygen or other electron acceptors such as NO_3 , SO_4 , ferric

- iron, which can stimulate microbial growth in the absence of oxygen,
- iii). adequate concentration of electron acceptors required,
- iv). natural groundwater circulation also influences the number of electron acceptors required in respect of providing enough mixing between contaminant water and surrounding water, and
- v). the presence of elemental nutrients such as nitrogen and phosphorus for microbial cell building.

Before implementing intrinsic bioremediation, the capability of indigenous microorganisms and site characteristic should be investigated thoroughly.

ii. Engineered bioremediation

Engineered bioremediation is aimed at accelerating the microbial activity using engineered site modification procedures such as well installation to circulate fluids or nutrients to stimulate microbial growth. The principle is to isolate and control a contaminated site so that it becomes an *in situ* bioreactor. The system is also called biorestoration and enhanced bioremediation. Engineered bioremediation is usually preferred due to time and liability.

The requirement of a site for engineered bioremediation is that the subsurface materials can transmit fluids. If contaminants occur in an unsaturated zone, or vadose zone, the remedial fluids are added in the gas phase whereas in groundwater remediation, the fluid can be circulated in water or by air injection.

A system which circulates water or groundwater should have a hydraulic conductivity greater than 10^{-4} cm/s. In an air circulation system, the intrinsic permeability should be greater than 10^{-9} cm². A site which has fractures or irregularities may give problems of channeling when distributing fluids.

2.3.3 Evaluation of *in situ* bioremediation

In the majority of cases, the complexity of contaminant mixtures, hydrogeological conditions, and competing abiotic mechanisms of contaminant loss make identification

of a biodegradation processes complicated. The evaluation strategy should be consistent, logical should rely on convergent lines of independent evidence taken from the site itself (Ritmann et al., 1994). The general requirement to demonstrate that bioremediation is occurring includes three factors:

- i). documented loss of contaminants from the site,
- ii). laboratory assays showing that microorganisms are able to grow in site samples which in turn indicates the potential to transform the contaminants under the expected conditions, and
- iii). one or more pieces of evidence that the biodegradation potential is realised in the field by detection of changes in reactants (e.g. oxygen and nutrients) and products (e.g. CO₂ or intermediate metabolites) that may be indicative of known metabolic processes (Madsen, 1991; MacDonald & Rittmann, 1993; Ritmann et al., 1994).

These evaluations are not only applied during the testing phase but also in the field or at full scale. James (1990) delineated the minimum requirement for technology demonstration in the field. This included not only technical issues but also others such as information to the public, health and safety and cost evaluations.

2.3.4 Protocols for bioremediation application

A screening protocol which can provide a feasibility study and remedial action information is needed to evaluate the viability and efficiency of a bioremediation process. Protocols are stated in a step-by-step procedure to avoid other interpretations. Protocols should be developed to evaluate the effectiveness of primary substrates, supplemental nutrients, electron acceptors and their mode of delivery. Rogers et al., (1993) divided screening protocols for bioremediation into 3 distinct phases. They are:

- i). site characterization to collect information about the contaminants and contaminated media and a feasibility study,
- ii). a treatability study should be performed to develop information on the effectiveness of bioremediation and to optimise process parameters, and
- iii). a design phase which considers contaminant removal rate and scale-up parameters.

i. Site characterization/feasibility study

This stage is required to evaluate whether bioremediation is a viable remedial technology. Information gathered should include factors related to chemical characteristics of the contaminants and the chemical, physical and microbiological characteristics of the site. Secondly, this stage also determines whether the bioremediation can be carried out *ex-situ* or *in-situ*.

ii. Treatability study

The primary objectives are:

- i). to evaluate rapidly and extensively the susceptibility of site soils to biological treatment, and
- ii). to determine the rate and extent of the level of treatment which can be achieved.

A treatability study can be carried out at all levels of process sophistication from a simple beaker test to a full-scale or a larger scale pilot studies. Rogers et al., (1993) however, suggested that a treatability study should be carried out in 3 phases. Phase I would be to determine if bioremediation is indeed an appropriate remedial technique whereas Phase II is intended to provide design criteria for a full-scale remediation project. Phase III would be the application of process at full-scale in the site.

Ritmann et al. (1994) allocated the kinds of treatability test needed according to its hierarchical goal. Level 1 to question if its biodegradation may be approached by a simple laboratory microcosm. Level 2 to evaluate kinetics, inhibition, sorption, etc. and requires laboratory microcosms including hypovials, shake flasks, columns, and slurries. Level 3 to evaluate if a site specific condition needs a field pilot study.

The protocols are based on the premise that chemical contaminants must first desorb and diffuse from soil and enter an aqueous phase before they can be assimilated by the microbes and degrade. The treatability study protocol is designed to evaluate equilibrium sorption relationships, sorption kinetics, and biological oxidation (Rogers et al., 1993).

Phase I of site characterisation includes:

- i). chemical analysis to identify chemicals of interest and their quantification,
- ii). microbial composition and enumeration in contaminated soil,
- iii). toxicity testing to predict any toxic substance which inhibits biodegradation, and
- iv). physical characterisation to gather information on its desorption and hydraulic capability.

Phase II which is that of testing involves:

- i). testing abiotic soil desorption to examine effectiveness of bioremediation due to contaminants' solubility ,
- ii). biological slurry reactor to gather evidence of microbial ability to biodegrade desorbed contaminants, and
- iii). pan microcosm which emulates land treatment process or column tests to determine the kinetics of reaction.

Following Phase II it is expected that the bioremediation technique can be started to be implemented at full scale or field scale.

To obtain more information on treatability tests, Baker and Herson (1994) summarised complete evaluation of available standard biotreatability test protocols and related them to many factors namely, microbial growth-inhibition tests, aerobic and anaerobic systems, toxicity testing, and quantification and monitoring biodegradation tests.

2.4 Leachate Treatment

2.4.1 Leachate characteristics

Leachate, which is generated from landfilling of wastes, varies widely in composition and quantity. The quantity depends on water input to the landfill and the climate. It can be from surface infiltration, liquid in the waste and the groundwater flow. Leachate generation is also influenced by the engineered design, the age of the waste, compaction of the landfill and composition of waste. Lining the landfill site with appropriate liners and landfill management can control the contamination by leachate of the groundwater and the surrounding environment.

The leachate problem may be minimised by good landfill management which may include controlling leachate production by proper containment, revegetation to increase evapotranspiration and contour grading. The leachate that is eventually produced however, has to be collected and treated to prevent any contamination of down stream flow.

2.4.2. Leachate treatment

Landfill leachate can be treated in combination with domestic waste treated separately, recycled back to the landfill, or used for irrigation. The treatment process could be biological, physical or chemical. Salim (1992) considered that the addition of leachate to a domestic waste treatment plant should be less than 5% of the total flow. Some researchers have found that at 2% leachate addition the biological process of domestic waste was not affected. A higher proportion could reduce the treatment performance, affect sludge separation and inhibit the biological process due to high organic, ammonia and trace metal content.

Recirculation of leachate back onto the landfill may have a beneficial effect as the solid waste could be degraded and stabilised more rapidly because of the changes in moisture content. This practice however, does not exactly solve the problem. It is only a short term solution (Sturken et al., 1991). Spraying leachate for irrigation was quite common practice in the past and was considered appropriate for weaker leachates. Some problems could arise for the vegetation and the aquatic environment if the leachate sprayed contained toxic and hazardous substances.

i. Biological treatment

Leachate from recent landfilling usually contains volatile fatty acids and high concentrations of dissolved organic matter which can be readily degraded by biological treatment. There are mainly two types of biological treatment, namely aerobic and anaerobic. Leachate treatment has been widely studied using either aerobic or anaerobic systems or a combination of both processes.

The aerobic process involves biological oxidation (respiration) and biosynthesis of organic matter by microorganisms in the presence of oxygen. Biological oxidation results in mineralization of organic matter. Biosynthesis converts organic matter into biomass which is then removed by settlement of excess activated sludge solids. The aerobic process at a treatment plant takes place with the biomass in suspension or attached to a support medium. Some examples of suspended growth systems are treatment by activated sludge, waste stabilization ponds, and aerated lagoons. Attached growth systems occur in biological or trickling filters, rotating biological contactor (RBC), BAFFs, etc.

The anaerobic process consists of three steps, namely hydrolysis and fermentation to produce acids, dehydrogenation, and methanogenesis. The microorganisms responsible for the processes are also classified into 3 groups. Acid forming bacteria (acidogenesis) in the hydrolytic and fermentation process convert organic waste into organic acids as intermediate products. The second group is the acetogenesis bacteria that are classified into bacteria which produce hydrogen and another group that consumes hydrogen. The acetoclastic methane bacteria use the acetate which is produced by the acetogenic bacteria. They, together with H₂-utilising bacteria produce methane as the final step in anaerobic treatment.

The constructed wetland process is a treatment technology which combines aerobic and anaerobic processes. Its application for treating many types of wastewaters has recently been widely investigated. A typical process includes the use of emergent macrophytes in a lagoon where the wastewater is distributed evenly allowing treatment by microorganisms in the root zone of the plants to occur. The technology offers simple design and operation and low capital cost although it requires a large area of land.

ii. Chemical and physical treatment

The requirement to achieve higher effluent standard of wastewater treatment has led to the development of chemical and physical processes. These processes include chemical precipitation, coagulation, ultrafiltration, microfiltration, ion exchange, reverse

osmosis, carbon adsorption and electrodialysis. The technologies are usually capable of removing colour, turbidity, specific hazardous substance, and heavy metals. Unfortunately, not all organic and inorganic substances are removed. Such treatment may be combined with biological treatment or other physical and chemical treatments to effectively remove the contaminants.

iii. Leachate treatment processes

The application of the above treatments for leachate management has been investigated by many researchers. Salim (1992) compared the use of aerobic RBCs, Upflow Anaerobic Filters (UAF), and Activated Carbon Adsorption to treat high and low strength leachates. He concluded that for high concentration the UAF performed better. The removal of COD in the RBC was 90% for low strength substrate with biological processes removing organic matter more efficiently. The remaining refractory organics were readily absorbed in the activated carbon column. The most effective treatment was a combination of biological treatment as the first stage, and activated carbon column as the polishing stage.

Leachate treatment using chemical processes has been reported by Fettig et al., (1996) who utilised preozonation and activated carbon adsorption. Kim et al., (1997) used Fenton's reagent (Fe(II) and H₂O₂) and ultra violet light. Papadopoulos et al. (1998) employed physico-chemical and bio-oxidation. Wetland systems have also been studied by Bulc et al., (1997). Nedwell and Reynolds (1996) successfully treated landfill leachate using methanogenic and sulphate reducing digestion. Nanofiltration and reverse osmosis for leachate treatment have been reported by Linde and Jonsson (1995) as an increasing practice in Europe (Franken & Fane, 1991).

2.4.3 Leachate treatment by reverse osmosis

The application of reverse osmosis has been traditionally used for the treatment of seawater or brackish water. Development of RO technology is now being considered for: feasibility study application in wastewater treatment, ultra-pure water, water softening, and other industrial wastewaters. Reverse osmosis offers advantages such

as separation of organic and inorganic compounds; it is relatively easy to design and operate; it is possible to combine with other technologies to improve separation and efficiency (Williams et al., 1992).

The application of reverse osmosis in industrial wastewater treatment has included: (Williams et al., 1992)

- i). chemical process industry: to reduce the discharge of hazardous wastes and the recovery and reuse of feedstock or products,
- ii). electroplating: to remove, recover or recycle heavy metals generated so that the process benefited and recovery of up to 95% on most metal could be obtained,
- iii). pulp and paper: to reduce wastewater quantities and to remove dissolved solids, colour and organics,
- iv). textiles: to reduce water volume as a water management system, remove salts, dyes, fatty acids, surfactants, scouring agents, oil and greases, oxidizing and reducing agents; and thermal recovery because membranes can tolerate temperatures of 55° to 85°C,
- v). petroleum industries; some contain inhibitors to microorganisms: RO removes water soluble low molecular weight compounds, organic and oil, and
- vi). power generation; RO can be used for brackish water desalination.

Sturken et al. (1991) reported that gaseous contaminants could also be treated using membranes. They recovered organic vapour from an off-gas stream using a modified plate and frame module made from a silicone-polyetherimide composite.

The application of reverse osmosis treatment for landfill leachate started from experiences in industrial wastewater treatment (Slater et al., 1983). Reverse osmosis has been shown to remove 91% TOC from a sanitary landfill leachate using a cellulose acetate and composite membrane (De Walle in Williams et al., 1992). A tubular cellulose membrane with lime coagulation as the pretreatment reduced 98% TDS, 68% COD and no sign of fouling was detected even at 75% water recovery. Hasbach (1995) found that the reverse osmosis cost was less than 1 cent/litre including capital cost. Linde et al. (1995) compared the use of membranes for conventional leachate and special leachate which was generated mainly from fly ash landfill. The study found that for the special leachate the concentration of salt and osmotic pressure were too

high and produced a low water flux. In contrast, RO removed 98% of COD and $\text{NH}_4\text{-N}$ from conventional leachate. They further noted that RO was more cost effective than activated carbon adsorption for toxic solute removal. Reverse osmosis was capable of removing large non-polar compounds, suspected to be organic chemical carcinogens from wastewater (Linde et al., 1995; Slater et al., 1983).

Although leachate has been widely studied in the application of RO membranes, its application relies on careful design and operation because problems such as fouling may be encountered due to inappropriate feed quality. Pretreatment can be used to minimise the fouling problems. The development of membrane configurations has resulted in landfill leachate being successfully treated using Rochem's disc tube without a membrane fouling problem. This was a high pressure RO which was designed to force the contaminated feed water flow in parallel rather than perpendicular (clead end). The flow pattern prevented contaminants from depositing directly on to the membrane surface resulting in the feed stream forming the brine concentrate. In this tube, 99.98% sulphate was removed (Anonymous, 1995). Similar results have been reported by Sturken et al. (1991) using disc tube modules in the largest known RO plant treating leachate. COD and TOC removal were greater than 98%; hydrocarbon was reduced by 97.8%; metals such as nickel, chromium, copper and vanadium were successfully decreased by greater than 95%.

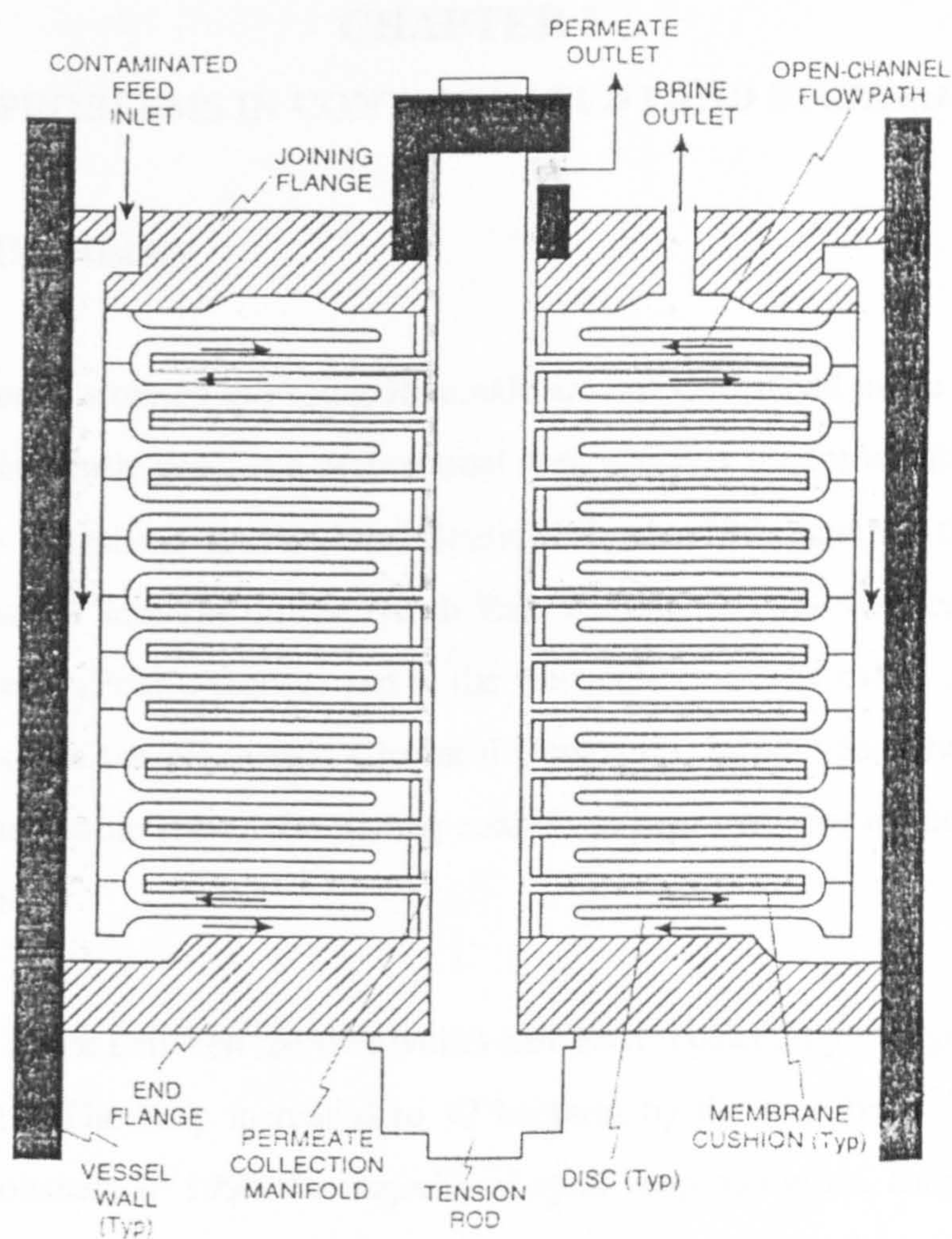


Figure 2.2 Cross-sectional drawing of Rochem's Disc Tube™ module
Source: Anonymous, The Hazardous Waste Consultant (1995)

CHAPTER 3

PROBLEMS IN CONTAMINATED LAND STUDIED

3.1 Land Use History

The area studied is situated in Felling-Riverside area of Gateshead in the North East of England. It is currently used as a recreational area which is predominantly grassed and is close to the Gateshead International Stadium. In the 19th century, the area was a centre of industrial activities in the North East and where two chemical works were located in the east (Friar's Goose) and in the west-side (formally called Allhusen's and which later became the Newcastle Chemical Company). In between, there was a ship building yard and some staiths for loading coal. A colliery was also located to the south west of the works.

The vast open space between the two works had been replaced by a large spoil heap by the end of 1916. The heap increased to 12 hectares by the time both chemical works had been demolished. In 1995 the majority of spoil heap had gone, leaving only a few mounds. The Friar's Goose Works has been replaced by open land, a small marina and a trading estate. Allhusen's was covered in part by the Gateshead International Stadium, and there is now a housing estate situated to the south of the former spoil heap.

3.1.1 Industries in the Gateshead area

i. Mining

Coal has been exploited from Gateshead to provide fuel for glassworks on the Tyne since the 17th century. By the 18th century it was also sent to London from the Tyne which was transported in wagonways to the River. There were 17 disused mine shafts. The most shallow mine recorded was some 70m below surface. The Tyne Main colliery, which was the most prominent mine was located to the south west of Friar's Goose Chemical Works. This still operated in the 19th century. A steam driven pump

was installed at the end of 18th century, to dewater the Tyneside Coal basin which allowed the exploitation of deeper coal seams in the area. This pumping station was closed in 1815 and the mines were flooded. As a result there is a mine discharge in the area onto land adjacent to the River Tyne which is alkaline and contains metals and sulphur.

ii. Chemical works

The Industrial Revolution in the 19th century boosted the textile, dye and glass industries which in turn increased the alkali industries in the area. Factories which manufactured soda, bleaching powder and soda crystal started to develop at Friar's Goose. A small factory which produced shale oil was also built a few years later. The glassworks which was located in the South Shore then expanded to have a saw mill and a soda factory. Due to lack of safety and poor working condition, these industries created major pollution problems. In addition, Belgian competition, and strict European and American tariffs led to the closure of glassworks. The alkali works was also closed in 1883 whereas Friar's Goose still continued until 1915.

iii. Ship building

Ships were built very near to Friar's Goose in the 1920s and the company expanded onto land at Friar's Goose after demolition of the chemicals works but closed in 1964.

3.1.2 Source of contaminants

i. Leblanc process

The Leblanc process was a technique used to produce soda and was originally developed in France. The process utilised sulphuric acid from pyrite and nitre. It also required coal and salt which were abundant in the Gateshead area. This made the Leblanc process a very popular practise. The nitre was imported and sulphuric acid was manufactured in each factory. A diagram of Leblanc process is shown in Fig. 3.1. Although the process in Europe was operated with the minimum wastage of raw

materials, this was not the case in Gateshead where abundant raw materials were available.

ii. Sulphuric acid production

Sulphuric acid was required in the Leblanc process. Every factory produced sulphuric acid from a mixture of sulphur and nitre in an iron dish over a pot of water and under a glass bell. The process was carried on chambers of lead with a steam jet and air passing over the surface. The aim was to purify the acid. The most important metal removed from the mixture was arsenic which was most likely dumped in the area. The two main processes involved sulphur hydrogen being agitated with the acid to allow the formation of arsenic sulphide (As_2S_3) or the addition of hydrochloric acid which produced arsenic chloride (AsCl_3). The impurities were then removed to leave a residual of purer acid for the Leblanc process (Keogh, 1997).

The process generated two unwanted by-products namely; calcium sulphide from the process of saltcake, coal and limestone, and hydrochloric acid from salt decomposition. Calcium sulphide could not be passed up a high chimney and it was therefore dumped onto land. The spoil heaps tended to be large since every tonne of soda generated 1.75 tonnes of calcium sulphide (Keogh, 1997). In the 1930s, there was a large smouldering spoil heap as a result of the calcium sulphide. It was estimated that a two millions tonnes spoil heap was generated which was still smouldering in 1951. Due to the value of nitre compound, recovery of nitrogen oxide was possible. The recovery itself released a gas stream that made the acid become more concentrated (ETC Report, 1995).

iii. Chance Process

The loss of sulphur in the waste heap was economically important. The recovery of sulphur was then attempted and resulted in the process introduced by Chance in 1882 (see Figure 3.2).

3.2 Pollution Problems Found in the Site

3.2.1 Contaminants in the ground/soil

The practice of dumping the calcium sulphide in the area around the works had been reported in 'The Penny Magazine' in May 1844 (ETC Report, 1996):

'The earthen waste is not thrown here heedlessly: it is laid in a compact form, having a smooth and level surface at the top; and if the memory of present things were to pass away, future geologists might be puzzled and conjecture how such a mound got there'.

To investigate the problems of the Gateshead contaminated land area, 11 boreholes were drilled on and around the old chemical waste tip (Phase I), and a further 26 boreholes (12-37) at 100m intervals to determine the extent of contamination and to facilitate groundwater monitoring. Another 19 boreholes (38-56) were required to delineate the region of the greatest ground contamination.

A preliminary survey of the 'Great Heap' was carried out in 1960 which showed chalk (calcium carbonate) to a depth of ten feet. Beneath the chalk was found a very deep black material which was thixotropic*. Red and yellow layers were also discovered. The black material was calcium sulphide waste from the soda extraction tanks. The yellow stratum was arsenic sulphide, and the red layer was iron oxide from the recovery of copper in burnt pyrites.

Analysis of soil/ground samples and leachate from the area showed that the soil contained a high concentration of inorganic carbon and sulphur compounds, i.e. calcium carbonate (white), calcium sulphide (black) and calcium sulphate (blue). The presence of organic sulphur compounds depended on pH, moisture content and oxygen concentration. Due to wastes containing caustic alkali, high pH was also found at the site. Some samples were shown to contain amines. Chemicals such as phenols with an

* thixotropic: becoming temporary liquid when shaken or stirred and returning to a gel on standing.

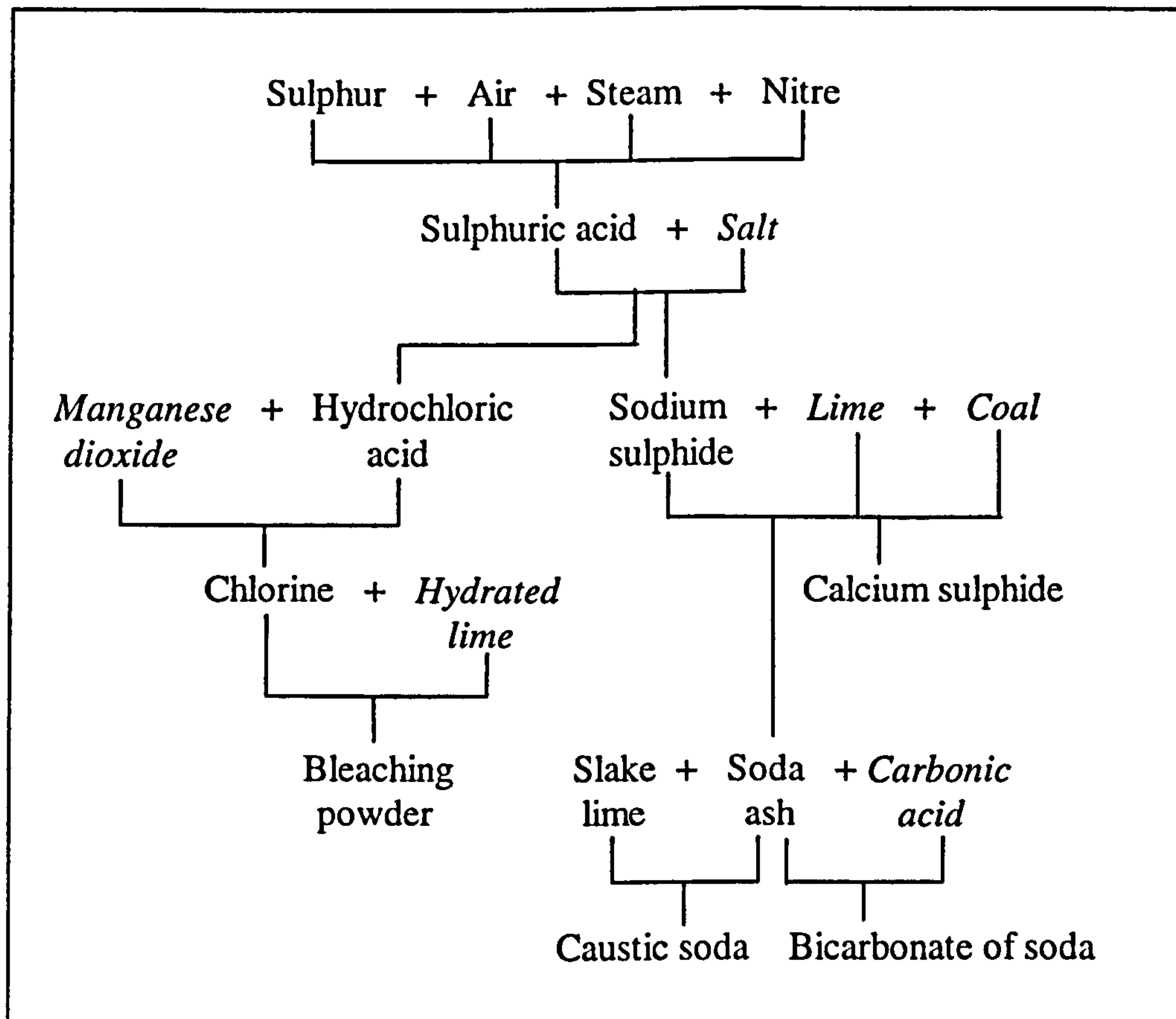


Fig. 3.1. Diagram of Leblanc process
Source: Keogh (1997)

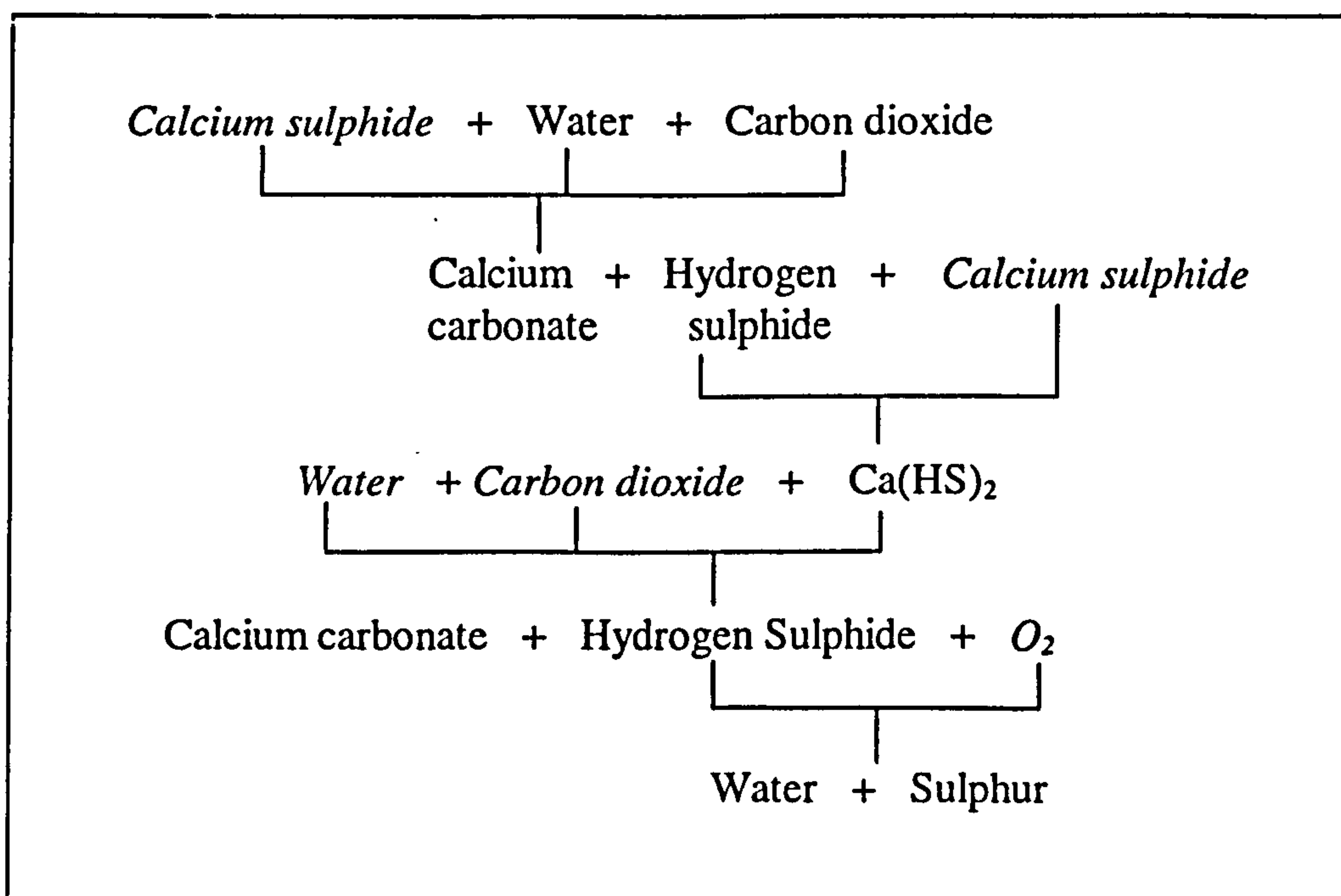


Fig. 3.2. Diagram of Chance Process
Source: Keogh (1997)

antiseptic oil were also detected. Some soil/ground samples were dry and powdery whereas others were thixotropic as a wet slurry (ETC. Report, 1995).

The measurement of conductivity of soil/ground samples showed that in the landscaped area it was 50-70 $\mu\text{mho/m}$ whereas in the waste tip itself it was found 70-100 $\mu\text{mho/m}$ indicating higher than normal levels of metals, sulphides or salts. During the reconnaissance study, the soil was found to have a variety of colours, from a grey/blue to green through black. These might due to decomposition of unwanted by-products of the caustic soda process which was predominantly inorganic carbon and sulphur compounds. According to ETC's report level of heavy metals were found with lead (Pb) having the greatest concentration followed by Hg, Ni, As, and Cr. Measurement of pH for 30 samples analysed showed it to be greater than 10. Sulphide concentrations in all samples were mostly greater than 5000 mg/kg whereas sulphate was found in half of the samples with concentration higher than 2400 mg/kg.

Consultants had also carried out laboratory and field permeability tests using the 'falling head method' on two boreholes samples which resulted in a hydraulic conductivity of 1.24×10^{-2} and 5.24×10^{-3} m/day (Head, 1992). Field permeability tests in some boreholes gave a range of permeabilities for each borehole of 4.32×10^{-4} to 5.07×10^{-2} m/day (ETC Report, 1996).

3.2.2 Contaminants in the leachate

The composition of leachate depended upon the nature of the solid waste dumped in the spoil heap or landfill, the surrounding earth and whether the decomposition was aerobic or anaerobic. Other factors such as the quantity of waste disposed, the time of storage, the degree of compaction, the amount of water in contact with the solid and also the temperature would have affected leachate composition.

The Consultants investigated some of the problematic outfalls which initially only affected a small area. Contamination however was considered to be spreading into a larger area, covering 25 hectares. Studies on the outfalls of the river bank showed that five discharged a coloured leachate often associated with hydrogen sulphide. This was

more prevalent after a period of heavy rainfall. The presence of hydrogen sulphide however, was dependent on the pH, and Eh, with a maximum production between pH 5-6. Analysis of the leachate started in 1991 and showed that the leachate quality improved with dilution after rainfall. The pH was found to be generally in the range of 6-8 and on one occasion a pH 4 was recorded. The sulphide concentration was around 2000-4000 mg/l. Arsenic concentration could be high (5 mg/l) though it was usually around 2 mg/l. Other trace metals detected included chromium, iron, lead, zinc, and nickel. The chloride concentration was around 250 mg/l (ETC. Report, 1995).

A small minewater discharge has been located close to the main outfalls at the site the quality of which was neutral pH, alkalinity, temperature around 10° C and low in iron. There was an unusually high sulphate loading in the wastewater which indicated the overlying spoil heap was influencing the mine water quality. It was however, thought that the majority of the water was of deep mine origin as the shallowest workings of the Tyne Mine Colliery were 70 metres below the surface (Keogh, 1997).

Summaries of chemical analysis of soil/ground and leachate samples from the consultants investigation are provided in the Appendix B.

CHAPTER 4

TREATMENT FUNDAMENTALS AND PROCESSES

4.1 Bioremediation Processes

4.1.1 Microbial growth in soil systems

Microbial growth can be defined as an orderly increase in the chemical constituents of organisms. An increase in total mass is not necessarily an indication of growth but may be due to synthesis and accumulation of cellular reserves. Growth normally results in cellular multiplication or an increase in population size. Evaluation of microbial growth is usually investigated from the size of biomass, their activity or diversity. The growth curve consists of a lag phase, an exponential phase, stationary and death phases. For some microorganisms, the growth curve can have a second lag phase after the first stationary phase, followed by the second exponential, stationary, and death phases. This second growth curve may be possible if the microorganisms find another type of nutrient after the first stationary phase.

Soil is usually an oligotrophic environment and is often nutritionally poor. Microbial growth is not evenly distributed with the growth being concentrated at sites of available nutrients, an appropriate gaseous environment and sufficient water supply. In addition, the location is normally associated with colloidal size ($< 2\mu\text{m}$) surfaces of clay and humic material where the ratio of surface and volume is high. The surface area of one gram of clay may be 20 to 80m² (Alexander, 1994). Soil also poses an ionic property whereby anions are repelled, cations are attracted and nutrients are concentrated. Microbial attachment to the soil surface encounters both attraction and repulsion forces as the cells carry negative charge at a soil pH of 6. The attraction or van der Waals force is approximately $F \sim 1/D^6$ (D =distance) whereas the soil particles and microorganisms repel each other with repulsion force of approximately $F \sim 1/D^2$ (Campbell, 1983). In general the microorganisms are located just above the surface at secondary minimum to conserve the energy.

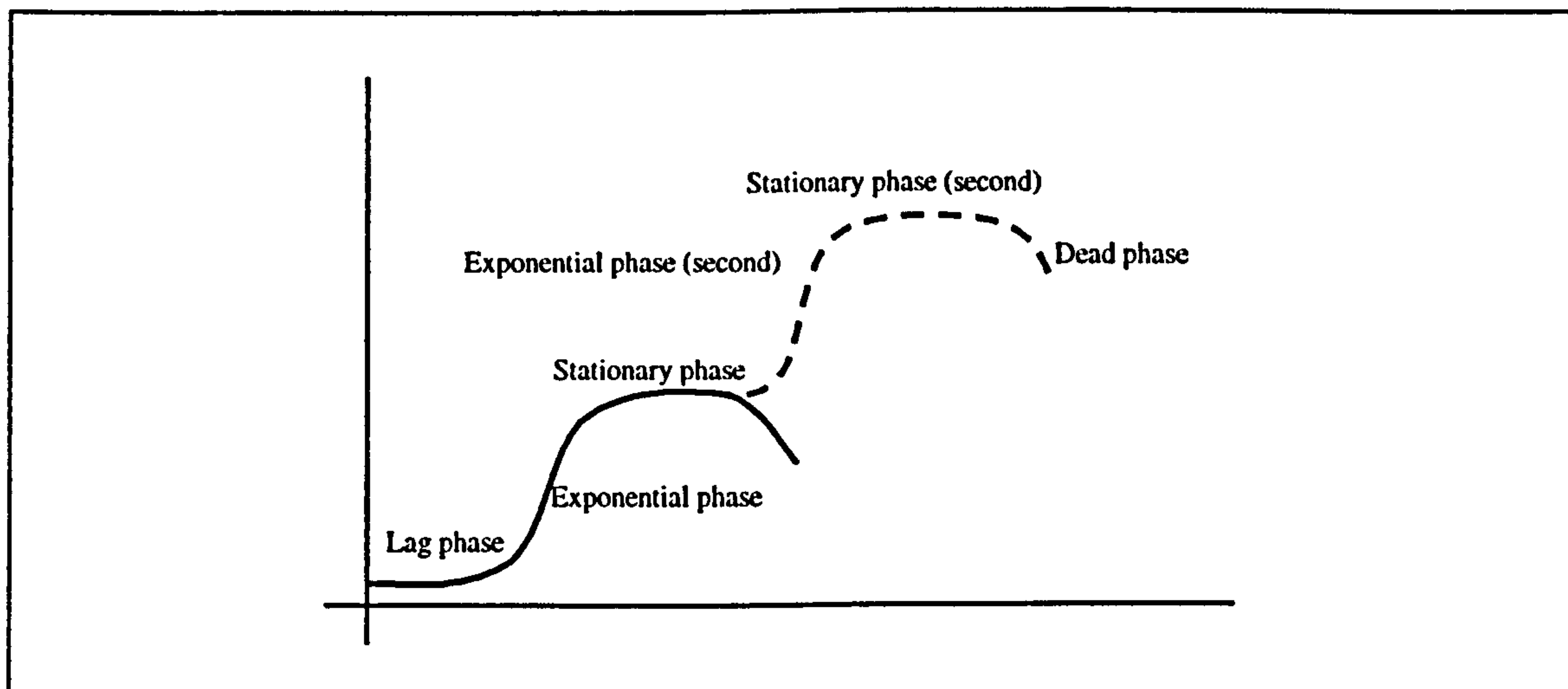


Figure 4.1 Growth curves

The assessment of microbial growth can be approached by determination of the cell mass or cell numbers either directly or indirectly. Cell mass is estimated directly by measurement of the dry weight of cells and indirectly using measurements of chemical components, enzyme metabolisms, radioactive, or densitometry measurements. Cell numbers are determined indirectly by plate counts or directly by direct counts either total counts or viable counts. This study only covered microbial growth assessment using the cell number estimation by total direct count analysis. Methods to assess microbial activity include measurements of respiration, microcalorimetry, ATP determination, enzyme activity, and thymidine uptake. The current study only employed microbial activity determinations using respiration products.

4.1.2 Microbial System for Bioremediation

The driving force of any biological reaction by microorganisms is the energy which can be obtained from several modes of metabolism. Metabolism manifests itself through oxidation-reduction reactions. Factors affecting metabolism are the energy source, the carbon source, and electron donors or acceptors that mediate oxidation-reduction. Oxidation is a process that removes electrons whereas reduction is the addition of electrons. The transfer of electron flow generates energy through a sequence known as the electron transport chain which is undergone repeatedly. The electrons are transported in a microbial system such as NADH (nicotinamide adenine dinucleotide phosphate). The electron acceptor establishes the energy yielding metabolism which is

usually calculated as ΔG or free Gibbs energy.

The metabolism is mainly classified into 2 categories, namely aerobic (in the presence of oxygen as electron acceptor) and anaerobic (in the absence of oxygen). Anaerobic metabolism utilises electron acceptors in successive levels from NO_3 , organic compounds, SO_4 , and CO_2 . These reflect denitrification, fermentation, sulphate reduction, and methanogen metabolism respectively.

Bacteria have developed a wide variety of respiration systems which are characterised by the nature of oxidants and reductants. The respiration of organic substrate by bacteria, however, is mostly consistent. The substrate is oxidised to CO_2 with successive removal of pairs of H^+ ions and electrons.

Another important concept of metabolism is cometabolism which is not an energy yielding reaction but the fortuitous transformation of a compound. Cometabolism is defined as the degradation of a compound only in the presence of an organic material that serves as the primary energy source. Hazardous chemicals may become a secondary substrate through cometabolism which is not fully transformed. Fortunately, the product of cometabolism transformation may be used as an energy source by other microbes. Consequently there may be competition for the enzymes between primary substrate and hazardous compounds. The final aspect of microbial metabolism is the recognition of preferential substrate degradation in which the highest energy yielding compound is degraded first (Cookson, 1995).

Energy yielding of an electron acceptor is the oxidation-reduction reaction in which the microbes obtain their energy. In the condition where there is more than one type of electron acceptor available, the microbes possess the ability to select the type of redox reaction which will give the highest energy. The amount of energy depends on the free Gibbs energy of substrates and products. The energy yielding level of electron acceptors is shown in Table 4.1.

The microbial redox reaction is motivated by a catalyst (enzyme) which is generated from the microbial cells. The catalyst does not change during the reactions but it increases the speed of the reaction since it lowers the activation energy of the redox reactions. The catalyst works very specifically to a chemical reaction. If some compounds are too large to pass through the cell membrane, the microbial cell secretes an extra cellular enzyme (exoenzyme) to digest the compounds.

In a bioremediation process the degradation of hazardous compound requires a specific metabolism. This can be controlled partially by obtaining the available electron acceptor. Other basic factors that can be manipulated in order to achieve successful bioremediation are temperature, pH, and inorganic nutrients. Temperature affects the composition and the function of the microbial communities which are responsible for degrading the hazardous compounds. The optimum pH is site and process specific. Many biodegradation processes change the pH due to the production of acids or bases. The buffering system should be sufficient or neutralization agents must be added to maintain an acceptable pH range. Inorganic nutrients such as nitrogen and phosphorus are essential for biological processes. They can be added as a variety of compounds, e.g. nitrate, ammonium salts, and organic compound such as urea. The choice is usually based on site geochemistry since it is possible for there to be an interaction with the phosphate and cations in water or soil. A treatability study is usually applied to determine the nutrient requirements (Anderson, 1995).

Table 4.1 Energy yielding levels in electron acceptor reactions

Type	pE	Reaction type
Aerobic:		
$O_2 + 4H^+ + 4e \rightarrow 2H_2O$	+20.8	Aerobic respiration
Anaerobic:		
$2NO_3^- + 12H^+ + 10e \rightarrow N_{2(g)} + 6H_2O$	+21.0	Denitrification
$NO_3^- + 10H^+ + 8e \rightarrow NH_4^+ + 3H_2O$	+14.9	Nitrate reduction
$CH_2O + 2H^+ + 2e \rightarrow CH_3OH$	+3.99	Fermentation
Formaldehyde Methanol		
$SO_4^{2-} + 9H^+ + 8e \rightarrow HS^- + 4H_2O$	+4.13	Sulphate reduction
$CO_{2(g)} + 8H^+ + 8e \rightarrow CH_{4(g)} + 2H_2O$	+2.87	Methane fermentation

Source: Cookson (1995)

4.1.3 Reduction-oxidation (Redox) potential

Redox potential is measured by a platinum electrode. The measurement of redox potential can be very useful in characterising the oxidation or reduction status of the soil. Oxidised soil has a redox potential of +400 to +700mV whereas a reduced environment possesses a redox potential of -250 to -300mV. The measurement of redox potential provides information on conditions that are favourable for increased bioavailability of heavy metals. Continuous measurements can also detect the beginning of reduction conditions as the oxidants are depleted.

4.2 Bioavailability of Contaminants in the Soil

Some biodegradable compounds may not be biodegraded for a number of reasons, including:

- i). the concentration of the toxic substance is too high for the microbes to metabolise,
- ii). one or more of the nutrients needed is too low to allow microbial growth,
- iii). the substrate may be at too low a concentration for organism multiplication, and
- iv). the substrate may not be in a readily available form for the microorganisms.

Consequently a study of the bioavailability of compounds is important as it may frequently account for its persistence. The unavailability of compounds may result from its sorption onto the solids environment or its entrapment within the physical matrix of the soil (Alexander, 1994).

4.2.1 Sorption into soil surfaces

The soil surface may dramatically change the indigenous microbial activity. These surfaces may alter the availability of organic chemicals, modify the pH and oxygen relationship, retain the microorganisms, or depress the extra cellular enzyme activity. As referred to at the beginning of this chapter (§4.1.1), the active surface may be clay minerals or humic substances of the soil. In addition, complex carbonaceous matter, or sometimes amorphous Fe or Al oxides or hydroxides may contribute as the active surface.

Many organic compounds are sorbed by the soils' constituents. This includes some amino compounds, organic phosphates, alkyl benzene sulphonates, cationic surfactants and certain high molecular weight materials. Factors affecting sorption of organic compounds are the types and concentration of solutes in surrounding solution, the type and quantity of clay minerals, the amount of organic matter in the soil, pH, temperature and the specific compounds involved (Alexander, 1994).

As mentioned in §4.1.1 adsorption may involve physical or van der Waals force, hydrogen bonding, ion exchange, or chemisorption (chemical bonding to the surface). Clay minerals and colloidal organic materials which are negatively charged may attract cations. Anionic organic compounds, in contrast may be repelled. As a result, the positive molecules at the pH value prevailing in nature are mainly retained by negatively charged surfaces. Large molecules may be retained on a clay surface by hydrogen bonding. The low molecular organic compounds are bound by ion exchange where an ion of one type in solution is exchanged for another ion that is on the solid sorbing surface.

The capacity of clays to affect biodegradation depends upon the clay types which in turn may be related to the cation-exchange capacity. Montmorillonite with a 2:1 ratio of Si and Al, for example, frequently sorbs microbial substrates because of its high cation-exchange capacity and its expanding lattice structure. Many organic substrates may penetrate between silica sheets and become fixed (Killham, 1994).

The organic portion of the soil is also responsible for the sorption of many compounds, particularly those which are hydrophobic. They are sorbed by native organic matter rather than the clay constituent of the soil. This is related to the octanol-water partition coefficient (K_{ow}) which expresses the hydrophobicity of chemicals and the percentage of organic C in the soil. The more organic matter present in the solid phase, the more hydrophobic molecule is sorbed. The hydrophobic molecule is retained in two ways. The first is due to physical sorption by the organic matter in which physical binding of the solute to organic solids occurs. The molecule is concentrated in the outer surface or within the solid pores and is then sorbed by physical or chemical forces. The other is

that the hydrophobic molecules diffuse and partition into the solid organic matter. This means that the molecule is distributed throughout the entire volume of the organic matter. These two mechanisms have created significantly different implication of the bioavailability of organic molecules to microorganisms. The first is more bioavailable compared to the second one.

As a result of adsorption, the substrate may be less or totally unavailable. On the other hand, biodegradation requires the compounds to enter the cell to react with the intracellular enzyme. Sorption may affect biodegradation for other reasons including:

- i). the rate of growth may be reduced if inorganic nutrients and growth factors are sorbed,
- ii). the microenvironment surrounding the surface may be less favourable for the transformation than the surrounding solution because the surface pH may be lower as these negatively charged surfaces concentrate H^+ from solution,
- iii). in contrast, sorption may also concentrate the nutrient at the surface of the absorbent or soil so that microbial growth is enhanced and biodegradation is stimulated, and
- iv). the microorganisms may be sorbed into the surface and make them associated more with the solids than the free liquid.

4.2.2 Desorption of organic compounds

Although sorption often reduces the microbial growth rate and the extent of biodegradation, but some microorganisms can utilise sorbed material as sources of carbon, energy, nutrients, and the compounds can be transformed. How the sorbed molecules become available to microorganisms, however, is not clear. Alexander (1994) proposed two hypotheses on the mechanism. The first was based on the concept that the organism utilised the chemicals which were initially in solution and metabolised the compounds in solution as a result of spontaneous desorption from the soil. The rate of metabolism depended on the desorption rate as this supplied the substrate in the soluble phase. Secondly, the microorganisms excreted metabolites that facilitated desorption so that the rate of biodegradation was greater than the rate of spontaneous desorption.

The other way to desorb hydrophobic compounds is by distributing a surfactant into the soil. A high concentration of surfactant may be needed to desorb the compounds and to bring them into aqueous solution. Studies by some researchers reported (Alexander, 1994) that at low concentration, two nonionic alcohol ethoxylate surfactants markedly enhance the rate of mineralization of phenanthrene and biphenyl which were sorbed to soil. Based on this, it is possible that surfactants produced by microorganisms at low concentration may facilitate the utilization of sorbed compounds by these organisms.

It is also possible that sorbed organic compounds are metabolised directly by the microorganisms that are attached at the same surface. The microorganisms come into contact with the compounds that penetrate into the cells without entering the surrounding liquid (Alexander, 1994)

The desorption process is particularly important for contaminated soil. It is required for the prediction of the fate and mobility of contaminants in order to develop a cost-effective remediation technology. Some contaminants, however, are considered persistent in soil as they are difficult to desorb. Readily desorbed contaminants, on the other hand, can be mobile and contaminate water supplies. This, however, could also be of potential use as decontamination can be carried out by leaching the contaminants.

4.2.3 Sorption of metal cations

Sorption of metal cations is pH dependent which is generally characterised by a narrow pH range. The pH range for metal cations is related to its hydrolysis or acid-base characteristic. In addition to pH, sorption of metal cations depends on chemical concentration, surface coverage, and the type of surfaces (Sparks; 1995). Selectivity is the relative affinity of a cation for a soil surface or adsorbent. This is affected by the proportion of cation, adsorbent, and solvent. The order of selectivity of monovalent metal cation depends upon the size of the hydrated radius in periodical table whereas for divalent metals, the selectivity is not as well defined.

4.2.4 Sorption of anions

The sorption of anions varies with pH. Sorption increases with pH until it reaches a maximum which is close to pKa. Adsorption is also sensitive to the ionic strength. Some anions can be sorbed in outer-sphere complexes such as for NO_3^- , Cl^- , and ClO_4^- . Most anions are sorbed as an inner-sphere complex such as molybdate, arsenate or phosphates. Outer-sphere complex is being electrostatically and differently attracted to a positive charge. The sorption is usually rapid and reversible and affected by ionic strength. In contrast, inner-sphere complex is sorbed at specific sites on the surface and form a chemical bond with the surface group. This inner-sphere sorption is usually slower and often not reversible and is weakly affected by the ionic strength of the aqueous phase.

In the case of SO_4^{2-} sorption, which is still considered puzzling some researchers concluded that SO_4^{2-} can be adsorbed as an outer-sphere complex. However, there is some evidence that SO_4^{2-} can be adsorbed as an inner-sphere complex (Sparks, 1995).

4.3 Biotransformation of Sulphur

4.3.1 Sulphur cycle

Sulphur is available in nature in both organic and inorganic forms. Its inorganic form exists in many oxidation states, ranging from +6 for SO_4^{2-} to -2 for H_2S (Spark, 1995; Killham, 1994). The oxidation state +6 is the most stable under aerobic condition. Sulphate is the predominant form of sulphur in aerobic waters and soils (Andreae & Jaeschke, 1992). Plants and microbes utilise sulphur in the form of sulphate. According to Killham (1994), the organic form of sulphur is available in well over 90% of sulphurs in non calcareous, non tropical, surface soils. About half of it is in the form of sulphate esters and esters with a C–O–S linkage and 20% is in the form of sulphur bonded to carbon as S-containing amino acids. The remainder is in a variety of inert organic forms. The C-S linkage occurs in amino acids such as cysteine, cystine, and methionine. This C-S form can account for 30% of the soil S (Sparks, 1995). 30 to

75% of the total soil S is sulphate esters which decreases with depth. Transformation of sulphur between organic and inorganic forms is entirely caused by the soil biota, particularly soil microbial biomass. They are important for both mineralization and also for the oxidation state of sulphur. The sulphur transformation process is shown in Figure 4.1.

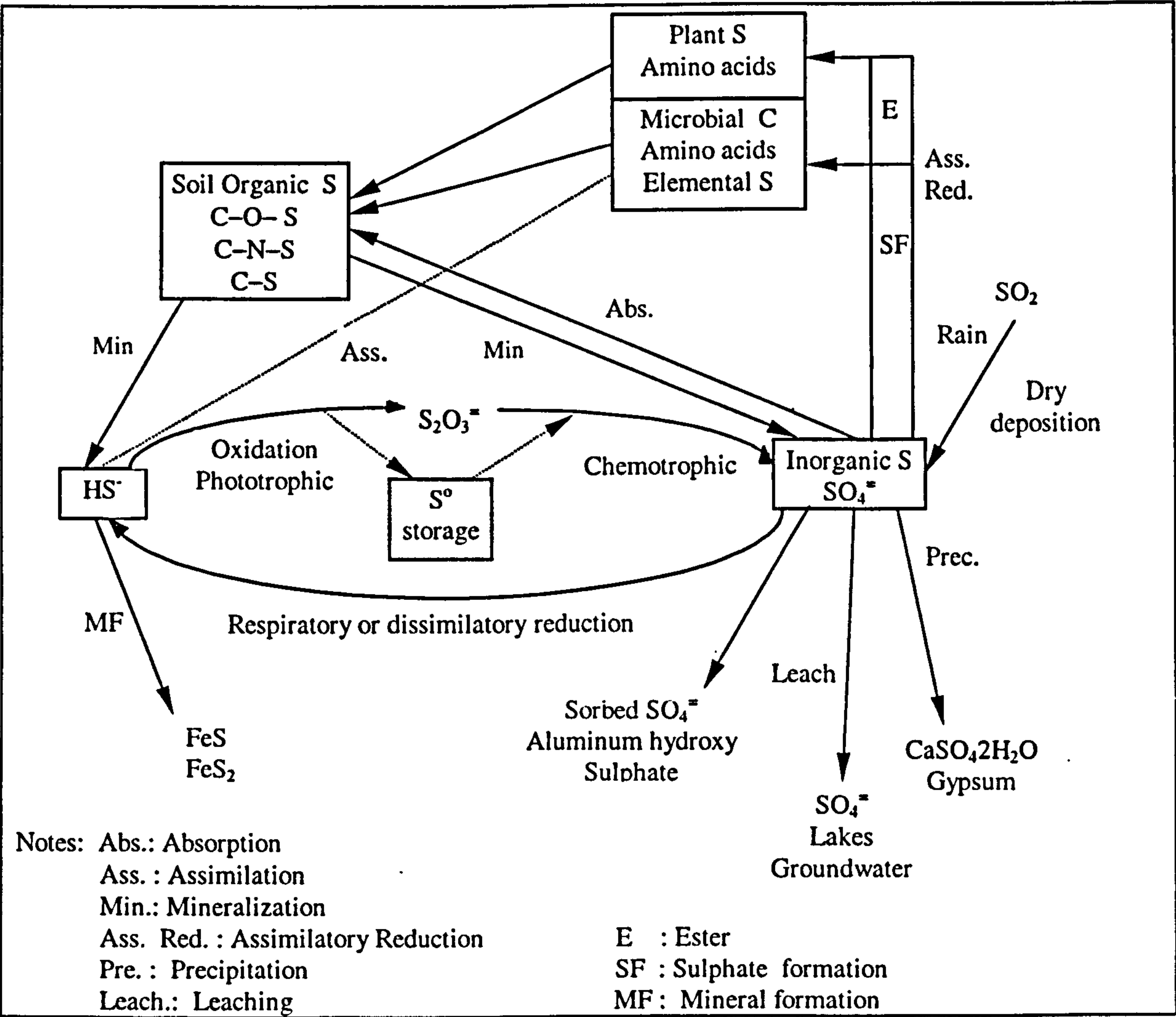


Figure 4.2 Sulphur Transformation in Nature
Source: Paul & Clark (1996)

4.3.2 Sulphur mineralization

Mineralization exists if microbes catabolise organic molecules thus releasing CO₂ and sulphur. Sulphur can be mineralised in two main processes namely biological and biochemical. Carbon-bonded sulphur is mineralised biologically when carbon is oxidised by soil microorganisms whereas non-carbon bonded organic sulphur is

mineralised through depolymerization (decrease the size of organic molecules) to be able to pass through membrane cells. The rate of S-mineralization in soil is influenced by similar factors which affect N-mineralization. These include water potential, temperature, pH, the presence of plants, drying or heating and the form and quantity of organic sulphur. During mineralization of amino acid-S, H_2S is rarely detected and mineralization of organic sulphur is measured as sulphate production.

4.3.3 Sulphur reduction

Because sulphate is available as the most stable sulphur form, its reduction to a more reduced form is necessary for the formation of volatile sulphur and emission to the atmosphere. The biochemical reduction of sulphate was considered as a driving force of the atmospheric sulphur cycle. In the global environment, where four compartments, namely atmosphere, biosphere, lithosphere and hydrosphere are inter-related, there are two types of biochemical pathway of sulphate reduction. These are dissimilatory and assimilatory sulphate reductions.

Dissimilatory sulphate reduction is strictly an anaerobic process. Heterotrophic microorganisms utilise sulphate as an electron acceptor to support their respiratory metabolism. The process is considered to be the major pathway of H_2S production. However, since the process is strictly anaerobic and poses a mixing barrier to avoid oxygen supply, the escaped H_2S gas is also limited. Most reduced sulphate becomes re-oxidised before leaving the sediment (Andreae & Jaeschke, 1992).

Dissimilatory sulphur reductions are mediated by anaerobes, organotrophic organisms that utilise low molecular weight organic acids, alcohols, and often H_2 as electron donors. They use sulphate or other inorganic sulphur forms as electron acceptors. The main genera of sulphate reducers are *Desulfotomaculum*, *Desulfovibrio*, and *Desulfobacter*. Killham(1994) considered that the soil ecology of sulphate reducers is dominated by three main factors:

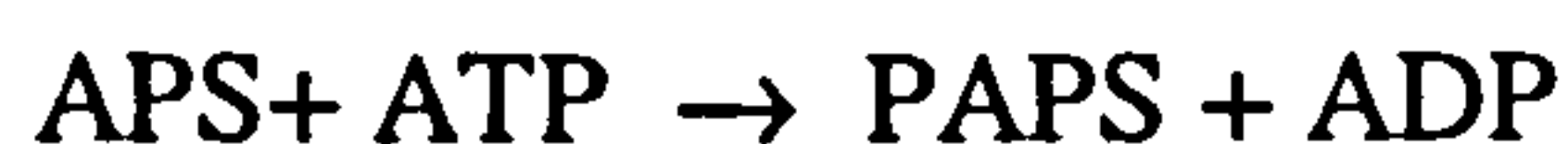
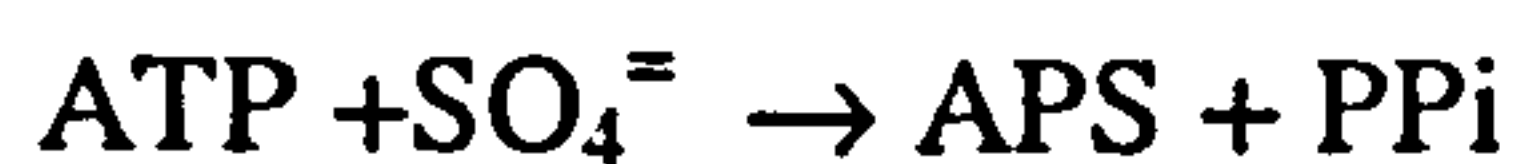
- i). sulphate reduction is generally restricted to water saturated soil where anaerobic conditions prevail (redox potential approximately -220mV at neutral pH),
- ii). sulphate reducers that are heterotroph, are controlled by the supply of organic

matter as their energy source, and

iii). sulphate in the soil solution should be adequately supplied.

Due to the low sulphate concentration in natural soil, the processes usually occur in soils which are affected by seawater, sulphate-fertilised soil, S-polluted soil, and soil affected by weathering of S-minerals (Killham, 1994). Soils supplemented with sewage or animal slurry are high in sulphide production.

Assimilatory sulphate reduction occurs in aerobic or anaerobic condition as a result of the action of microbes and plants. The sulphur is converted into sulphur-containing amino acids and other organic compounds in microbial protoplasm. The sulphur becomes unavailable for plant uptake and is said to be being immobilised. The assimilation process involves sulphate ion activation as a two-stage process which leads to the production of energy-rich sulphate nucleotide (ester) APS (adenosin 5'-sulphato-phosphate) and PAPS (adenosine 3'-phosphate, 5'-sulphato-phosphate). The reaction can be describes as follows: (Killham, 1994)



The overall reaction is:

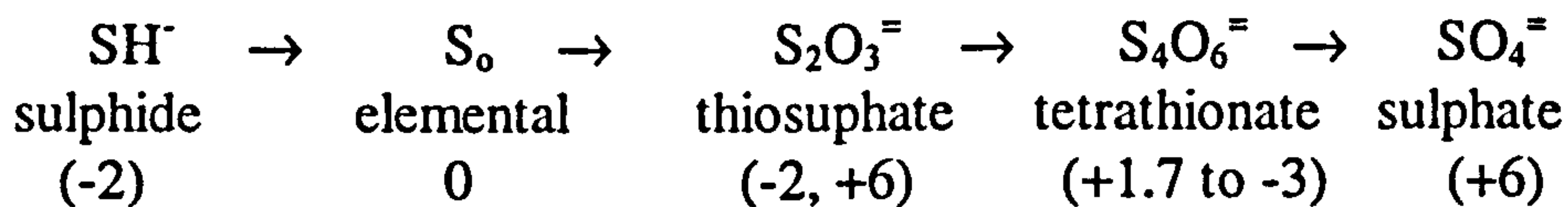


which is the route of organic sulphate formation. The nucleotides are then used for the synthesis of S-containing amino-acids.

The balance of mineralization and immobilization in soils depend on the concentration of readily usable sulphur in organic residues relative to the utilisable concentrations of carbon and nitrogen. A C: S ratio of 400:1 (\cong 0.1% S) results in immobilization whereas at a C:S ratio of 200:1 (\cong 0.2% S), sulphur is released into the environment. Between these two levels, microbial growth occurs without mineralization or immobilization (Paul & Clark, 1996). Killham (1994) stated that the critical C: S ratio at which immobilization exceeds mineralization was in the range of 200:1 to 400:1. However, they agreed that immobilization is encouraged by widening the N: S ratio as sulphur mineralization is more highly correlated with mineralization of N than degradation of C.

4.3.4 Oxidation of inorganic sulphur

The oxidation of inorganic sulphur, which changes the valence states of sulphur, can be accomplished both chemically and biologically.



The oxidation can be carried out by diverse microbial groups in soils. The process involves phototrophic or chemotrophic-S bacteria. Phototrophic bacteria are divided into two groups based on pigmentation, the green and purple sulphur bacteria which include cocci, vibrios, rods, spirals, budding, and gliding forms. The purple groups include *Beggiatoa*, *Thiobacillus*, and G^- chemoorganotrophs such as *Escherichia coli*. The green S bacteria are from the family of *Chlorobiaceae*. The most common chemotrophs are the *Thiobacilli* which are divided into groups with neutral and acidic pH values.

There are also heterotrophic bacteria that are able to oxidise inorganic sulphur, such as *Arthrobacter*, *Bacillus*, *Micrococcus*, *Mycobacterium*, and *Pseudomonas*, some actinomycetes, and numerous fungi. Heterotrophic bacteria are believed to be the main oxidisers of sulphur in neutral and alkaline soils (Paul & Clark, 1996).

Due to the large variety of sulphur oxidiser microbes, the factors controlling the oxidation vary between one and another. The amount of sulphur available, soil moisture, and temperature are the most significant factors which affected the sulphur oxidation rates (Killham, 1994).

4.3.5 Sulphur volatilization

Sulphur volatilization occurs if volatile organic sulphur compounds are released from soils that contain decaying plants and animal residues, and in waterlogged condition. Biodegradation of amino acids containing sulphur can result in the production of

volatile organic sulphur such as methane thiol, CH_3SH , and dimethyl sulphide, CH_3SSCH_3 . These compounds produce a strong and unpleasant odour. Their formation, in addition to that of H_2S , accounts for the odour associated with sulphur-containing organic compound degradation (Manahan, 1994). Sulphur can be released as mercaptans, methyl and ethyl sulphides, thiols and other varieties of volatile organics (Killham, 1994).

4.4 Heavy Metals Immobilization in Soils

4.4.1 Microbial immobilization

Biological treatment for metal remediation is still in its infancy. Although some metals are essential micronutrients, they are toxic and can inhibit biological activity. The toxic effects were concentration dependent. However, certain microorganisms are capable of protecting themselves by adsorption, oxidation, reduction, or methylation mechanisms. This ability can be manipulated in order to reduce metal contamination. Technologies which have been studied to remediate metals by biological metabolisms include biosorption, bioleaching and bioextraction, biobeneficiation and biological oxidation or reduction (Smith et al., 1994).

i. Bioaccumulation

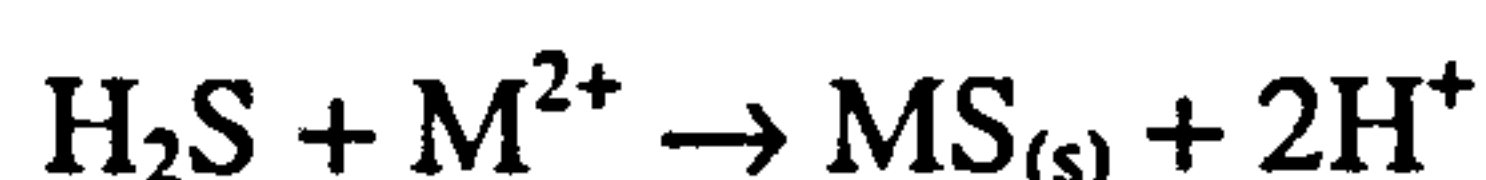
In remediation of metals, biological activity can be exploited to alter the chemical state, form, and distribution of metals. Bioaccumulation involves the transfer of metals from a contaminated matrix into biomass. Metals can be adsorbed onto certain living microorganisms or onto inactivated nonliving biomass. Microbial biomass has been shown to adsorb organic or inorganic compounds from the aqueous phase (Smith et al., 1994). The mechanisms of metal removal can be divided into two ways which involve the use of active or dead cells. In living cell utilization, the metals can be accumulated, adsorbed and taken up as a side effect of normal metabolic activity of the cells. The metal is not metabolised but concentrated through mechanisms such as ion exchange and complexation on the cell walls, intra and extracellular precipitation, and intra or extracellular complexation. For dead cell utilization, inactivated biomass

adsorbs metal into the ionic group on the cell surface or in the polysaccharide coating that is found in most bacteria. The binding of metals can be by utilising exchange of functional groups (such as carboxyl, phosphate residues, S-H or hydroxyl groups (Smith et al., 1994).

ii. Oxidation or reduction

Remediation of heavy metals can also be attempted by oxidation/reduction of certain microorganisms. The reaction may be carried out directly by the microorganisms or may be as a result of a reducing agent produced by the microorganism. Biological oxidation of metals has been commonly applied for mercury, cadmium, arsenite, iron(III), iron(II), manganese, and antimony. The metals that can be reduced include arsenic, ferric iron, mercury(I) and mercury(II). Oxidation/reduction reactions increase metal mobility in which insoluble metals become soluble. The metal is then collected for further treatment. This process is similar to soil washing or chemical leaching. On the other hand, the oxidation/reduction reaction may also reduce the mobility or toxicity of metals.

Metals can also be transformed indirectly by a microbial process as a result of a sulphate reducing mechanism that produces hydrogen sulphide and alkalinity. The process requires strictly anaerobic condition, a source of carbon, a source of sulphate, and a sulphate reducing bacteria population. The H₂S can react to precipitate metals as insoluble metal sulphides. The alkalinity production increases the pH which results in metal removal through the formation of insoluble metal hydroxides or oxides. *In situ* treatment of metals by this process generates metal precipitation and prevents migration in contaminated soil or groundwater (Smith et al., 1994). The reactions involved are:



iii. Methylation

This reaction refers to a process in which organisms attach a methyl group ($-CH_3$) to an inorganic form of metal. The methylation process produces organo metallic compounds which are more volatile than elemental forms. The metal is then removed by volatilization and collected from the gas stream. Methylated forms of metal are more mobile and may pose further contamination potential to the environment. Metals which can be microbially methylated include mercury, arsenic, cadmium and lead.

iv. Technologies of metals biotreatment

Biosorbents using active or inactive biomass are commercially available. Examples of biomass material include inactivated algae and natural biopolymer, chitosan, made from shellfish wastes, caustic treated killed bacteria, peatmoss *Spirulina* and others (Smith et al., 1994). Living biomass for biosorption has also been studied by many researchers in recent years. Metals removed by biosorption include cadmium, cobalt, copper, and mercury.

Bioreduction of mercury salts to metal has been investigated at bench-scale level to recover mercury (Mattison in Smith et al., 1994). The process may be carried out in bioreactors using tested organisms such as *Pseudomonas putida*, *Thiobacillus ferrooxidans*. Bioreduction of Cr(VI) to Cr(III) has been studied using indirect sulphate reducing bacteria or direct metabolism by pure strain or bacterial consortia. The direct metabolism process however, proceeds slower than indirect reduction.

Metal remediation using a wetland environment utilises aerobic process at the surface and anaerobic at a zone below the surface. Oxidation and reduction are the main processes in both zones.

Bioleaching is the microbiological solubilization of metals from a solid or semi solid matrix to improve removal. The technique can be used to recover metals from *in situ* or from excavated material. Metals are dissolved by microorganisms either by direct reaction, indirect attack by one or more metabolic products or by a combination of

both. The bioleaching process was originally used in mining practice and is now applied for spoil heap and *in situ* leaching.

Biobeneficiation is a process where the microorganisms improve the physical separation of a contaminated solid matrix into rich-contaminant and poor-contaminant streams. The process produces mineral concentrate and a tailing stream without substantial chemical changes of the processed matrix. Physical beneficiation occurs after excavated material is processed to reduce the particle size. The separation can be based on physical or chemical properties such as colour, particle size or shape, specific gravity, magnetic permeability, inductive charging, or surface chemical. An example of the technology is found in a flotation process to separate complex sulphide ore using *Thiobacillus ferrooxidans* that is able to modify the ore surface and improve the flotation process.

Metal removal can also be exploited using vegetation which can concentrate metals by taking them up through their root system and depositing the metals in the leaves (Smith et al. 1994; Pulford & Black, 1998; Kerr et al., 1998).

The biotreatment technology for metal removal can be selected after considering factors such as type and concentration of metal, matrix, pH, temperature, oxygen concentration, alkalinity, substrate availability, nutrient concentration, indigenous microorganisms, population density and the use of active or inactive biomass. The application of the technology has also to consider site conditions, volume of contaminated material, depth of contaminant, and site controllability.

4.4.2 Chemical immobilization

Although metal removal by chemical immobilization was developed earlier than biotreatment, the technologies available are usually expensive. The choice generally includes excavation, transport, and disposal in a landfill or excavation, treatment, and disposal in a landfill. Treatments involved are soil washing, followed by pH adjustment and precipitation.

The chemical addition process was divided into two main objectives, namely, binding the metal in the soil matrix (metal retention by soil) and leaching or removal of the metal from the soil (Tutem et al., 1998; Allen and Chen, 1993). Some chemical which usually used for these purposes include acids (HCl, H₂SO₄, acetic acid, etc.), bases such as NaOH, and chelating agents (EDTA, citric acid, etc.). Legault et al. (1993) used a hybrid of polymer to bind arsenic which is then separated by a membrane. Kiefer and Holl (1998) removed heavy metals using complexing agent in order to dissolve heavy metals from soil whereas Petruzzelli et al. (1998) immobilised the metals using the addition of paper mill sludge to increase sorption of heavy metals into soil matrix.

Czupirna et al. (1989) proposed an *in situ* immobilization by adding natural or synthetic chemical to the soil. The heavy metals should be neither hydrolysed nor desorbed under exposure of varying soil conditions. On the other hand, the chemical added should be resistant to chemical and microorganisms degradation over a long period of time. In addition, the chemical should not leach any toxic and organic or inorganic substances that may contaminate the groundwater. They compared 21 chemical additives which were classified into strongly adsorbing and weakly adsorbing chemicals. The strongly adsorbing insoluble chemicals which were added and distributed in the soil would not migrate down the soil and groundwater. Heavy metals were adsorbed, complexed, and/or chelated on to the additives. The weakly sorbing chemical addition caused the metals to precipitate or complex and/or chelate and then attach themselves onto the soil structure.

They found that Valfor 200 and lime could immobilise Cu, Ni, Cd, and Zn on their own or in combination. Valfor 200 is a sodium aluminosilicate that has a high sorption capacity for divalent and trivalent heavy metals. Czupirna et al. (1989) concluded that contaminant metal immobilization resulted not only from an ion exchange consideration but also strong covalent bonding was responsible for precipitation and chemisorption of metal contaminants.

4.5 Reverse Osmosis

4.5.1 Principle of RO

Osmosis is a natural phenomenon in which water passes through a semi permeable membrane from a lower solute concentration to a higher concentration until the equilibrium chemical potential is maintained. The pressure difference between two sides of a membrane at equilibrium is equal to the osmotic pressure. Reverse osmosis is a reverse flow from a higher concentration to lower side of concentration after applying pressure which is greater than the osmotic pressure. The process can be explained as shown in Figure 4.3.

According to the pressure applied, reverse osmosis can be classified into three categories, namely: high pressure RO (5.6 to 10.5 MPa); low pressure RO (1.4 to 4.2 MPa), and nanofiltration (0.3 to 1.4 MPa) (Bhattacharyya & Williams, 1992a). The term low pressure, however, is not consistent between researchers. Siler and Bhattacharyya (1985) mentioned 1MPa to 3MPa as low pressure; Sun et al. (1995) used LPROM at 830 kPa; Ujang & Anderson (1996) applied 670 kPa to remove heavy metals from electroplating waste.

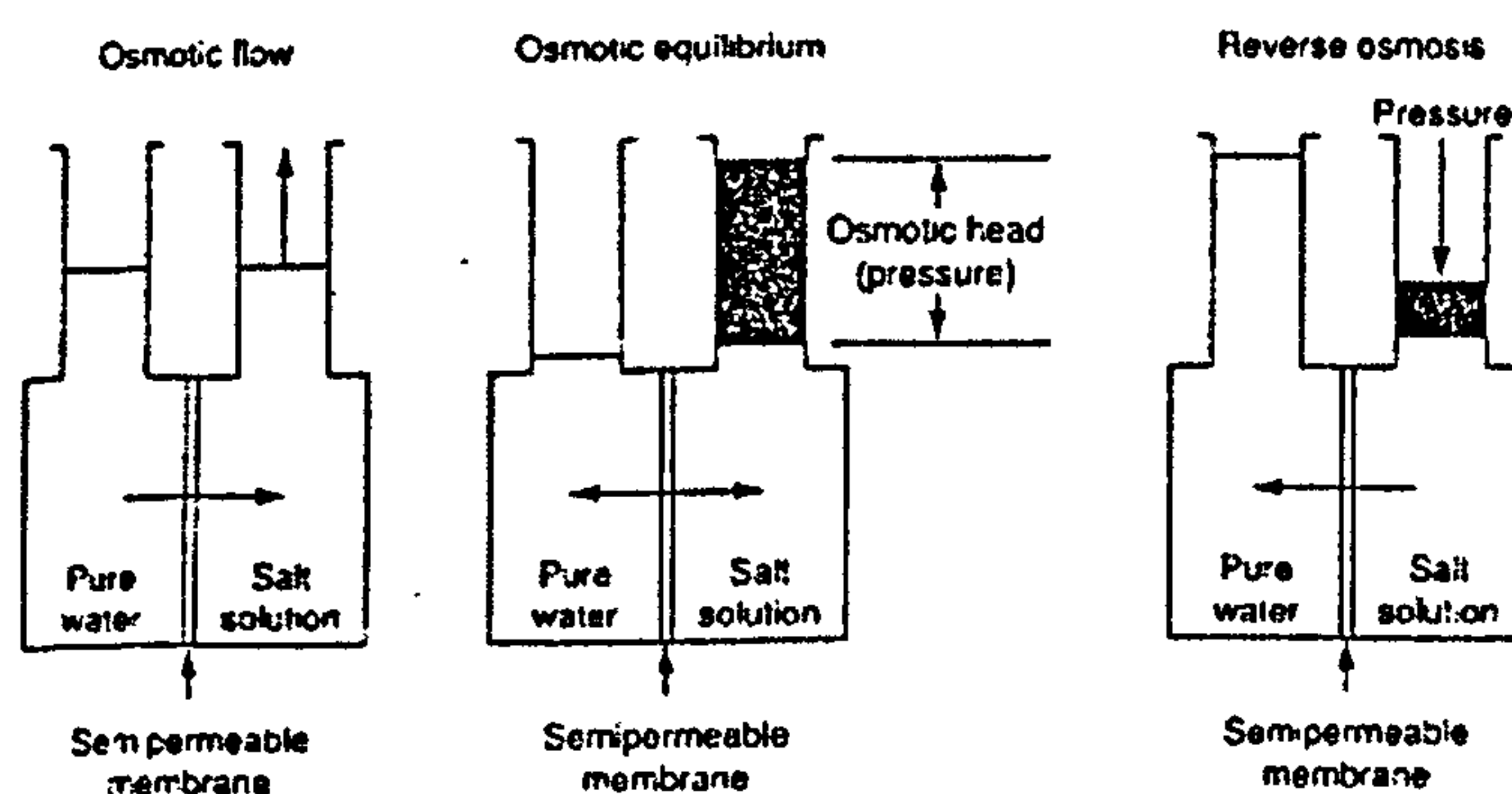


Figure 4.3 The reverse osmosis process
Source: LaGrega et al. (1994)

The rate of water transport across the membrane depends on the membrane properties, the solution temperature and the difference in applied pressure across the membrane. At constant temperature, water transport in a semi permeable membrane can be described by the following equation (LaGrega et al., 1994) :

$$J_w = (D_w C_w V_m / RT \Delta Z)(\Delta P - \Delta \pi) \quad (4.1)$$

where:

J_w = water flux through the membrane (gmol/cm².sec)

D_w = diffusivity of water in membrane (cm²/sec)

C_w = concentration of water (gmol/cm³)

V_m = molar volume of water = 0.018 l/gmol

R = gas constant = 82.057 (atm. cm³)/(gmol.K)

T = absolute temperature (K)

ΔZ = membrane thickness (cm)

ΔP = pressure differential across the membrane (atm)

$\Delta \pi$ = osmotic pressure differential across the membrane (atm)

$D_w C_w V_m / RT \Delta Z$ is defined as water permeation (W_p)

The osmotic pressure depends on solute concentration, solution temperature and the types of ion present. For dilute solutions, the osmotic pressure is calculated using the van't Hoff equation:

$$\pi = v_i c_i R T \quad (4.2)$$

where

π = osmotic pressure (atm)

v_i = molar concentration of the solute (gmol/l)

c_i = number of ions formed if the solute dissociates

R = gas constant (0.082 atm.l/gmol.K)

T = absolute temperature (K).

The membrane process produces a permeate (the portion of the feed that passes through the membrane) and a retentate or concentrate (the portion of feed that does not pass through the membrane). Evaluation of the membrane process usually involves observed solute rejection or salt rejection, water flux and water recovery. Solute rejection - R is defined as the percentage of salt in the feed that passes through the membrane into the permeate.

$$R = (1 - C''/C') \times 100 \quad (4.3)$$

where

C'' = concentration of solute in permeate (gmol/l)

C' = concentration of solute in the concentrate (gmol/l)

The water recovery is the percentage of the feed water that is converted into permeate and is described as follows:

$$r = Q_p/Q_f \times 100 \quad (4.4)$$

where r = % recovery or conversion

Q_p = flow rate of permeate ($\text{l}/\text{cm}^2\cdot\text{hr}$; l/hr)

Q_f = flow rate of feed.

4.5.2 Factors affecting membrane performance

Membrane performance is affected by recovery, temperature, pressure, compaction and concentration polarization (Brandt et al, 1993; Bhattacharyya & Williams, 1992a). Excessively high membrane recovery creates a high concentration of concentrate which reduces the permeate and increases salt passage. This may lead to membrane fouling or scaling due to the precipitation of sparingly soluble salt from the concentrate. Temperature changes the osmotic pressure and solute permeability and as a consequence will affect the water flux. Brandt et al. (1993) stated that a one degree Celsius increase in temperature will increase the membrane capacity by about 3%. Bhattacharyya and Williams (1992a) noted this increment of water flux as described by an Arrhenius temperature dependence on pure water permeability. At higher solute concentration the water flux becomes lower as the osmotic pressure of the solution is higher. Increasing the pressure produces an increase in water flow per unit area of membrane. With regard to salt passage, increased water flow results in a lower permeate salt concentration.

After some period of operation, the water flux can decrease with time as a result of membrane compaction. This is caused by creep deformation of the polymeric membrane over time (Brandt et al., 1993). The degree of compaction depends on the membrane material, applied pressure, and temperature. The tendency to creep is greater if the pressure and temperature is higher.

Concentration polarization is defined as the accumulation of membrane rejected solute at the membrane surface where the solute concentration is much higher than that of the bulk feed solution. Effects of concentration polarization are:

- i). decrease in water flux because the osmotic pressure at the membrane surface is increased,
- ii). increase in solute flux across the membrane,
- iii). precipitation of the solute if the surface concentration is higher than its solubility limit, leading to the membranes' pores plugging and reducing water flux,
- iv). changes in separation properties; and
- v). fouling or accumulating of material at the membrane surface, plugging the membrane pores and reducing water flux.

To reduce the possibility of concentration polarization, the feed solution close to the membrane surface should be maintained with good mixing. This can be done by modification of the membrane module which promotes turbulence in the feed channel or by increasing the feed flow rate so that the velocity is also increased.

4.5.3 Membrane materials and modules

Separation in membrane systems is governed chemically by the nature of the membrane polymer and physically by the structure of membrane. The first membrane material was called asymmetric cellulose acetate which was developed by Loeb and Sourirajan in 1963 (Bhattacharyya et al., 1992). An advanced membrane was later developed into a noncellulosic reverse membrane which is more resistant to pH and temperature changes.

Ideal characteristics of a membrane include: high water flux, high salt rejection, tolerant to chlorine and other oxidants, resistant to biological attack, resistant to fouling by colloidal and suspended matter, low cost, easy to form thin films or hollow fibres, strong (tolerant to high pressure), chemically stable, able to operate at high temperature (Brandt et al., 1993). During the development of membrane technology the most important material were cellulose acetate, aromatic polyamide, and thin film composites.

Cellulose Acetate (CA) was made from cellulose diacetate and cellulose triacetate formulations. The dense skin was a layer of 0.2 μ m on the porous layer which

developed an overall thickness of 100 μm . Increasing the acetyl content will increase salt rejection and chemical stability but the flux decreases. CA is poor in chemical stability and not capable of dealing with pH and temperature changes. The temperature range is between 0° to 30°C and the pH range, 4.0 to 6.5 (Brandt et al., 1993).

An Aromatic Polyamide (Aramid) was developed by Du Pont by solution spinning to form a fine hollow fibre. A dense skin on the outer surface was approximately 0.1 to 1.0 μm thick and the porous supporting structure around 26 μm thick. Aramid has an excellent chemical stability compared to CA. The temperature range is 0° to 35°C and pH in the range of 4 to 11 and is more resistant to biological attack. The disadvantage of Aramid membranes is their susceptibility to chlorine.

The Thin Film Composite (TFC) membrane was first introduced in the 1970s. The membrane comprises of an ultra thin barrier layer of 0.2 μm which is formed on the surface of microporous polysulphone that has been supported onto a porous layer. The advantages of TFC is its greater chemical stability, higher flux, and high salt rejection at moderate pressure, and it is resistant to biological attack. TFC can be operated continuously in a temperature range of 0° to 40°C and a pH range of 2 to 12. However, TFC has low resistance to chlorine and other oxidants.

The application of a RO process very much depends on its cost and the efficiency of the membrane packaging. A desirable membrane module provides safe operation at high pressure, no internal or external leaking, easy to flush and clean, minimal pressure drops, resistance to corrosion, and reliable for long term operation (Brandt et al., 1993).

The first membrane module was a plate and frame or tube-in-shell configuration. Now advanced membrane packages also include spiral wound, hollow fibre and tubular.

Spiral wound membranes are made from flat film membranes which are wound around a perforated polyvinylchloride or polypropylene centre permeate tube. Two or more leaves (membrane sandwich) are attached and wound round the centre tube. The

membrane sandwich is equipped with a product water channel spacer in between and a feed channel spacer. After winding, the outside is wrapped with tape or fibreglass for mechanical strength. The unit is around 30 to 150 cm long with a diameter of 2.5 to 30 cm. In operation some membranes are placed in series to meet the percentage of recovery. Spiral wound membranes have good resistance to fouling, are easy to clean, have an easy field replacement, and are available in a wide variety of materials. However, they have the disadvantages of a tendency for concentration polarization, moderate membrane surface to volume ratio, and difficult troubleshooting in multi element units.

Hollow fibre bundles are formed by orienting the fibres parallel to a perforated centre feed tube. The pressure is applied from the centre and flow is radially around the outside of the fibres. The advantages of hollow fibre membranes are their high membrane surface to volume ratio, high recovery in individual units, easy trouble shooting and easy to change the bundles. Disadvantages include sensitivity to fouling by colloidal and suspended material and they are not available from wide a manufacturer base or range of materials.

A tubular membrane module can contain up to 30 tubes and can be up to 60m in length. The membrane is normally supported within stainless steel. In the tubular design, the feed channel and permeate channel can be easily cleaned which make it suitable for food or dairy products. It also has a larger diameter giving more turbulent flow so that the membrane is very resistant to fouling. However, the module requires a high energy to provide pressure in large channels and has a higher capital cost due to low density packages.

This current study used a spiral wound module of sulphonated polysulphone at low pressure. Low pressure reverse osmosis membranes (LPROMs) have an economic advantage due to their lower energy requirement. Although it is not a new concept, recent developments in membrane technology enable LPROMs to produce a satisfactory volume of high quality permeate at low pressure. Sulphonated polysulphone is known to be superior to CA with respect to being highly chlorine resistant, has a high permeate flux and has good salt rejection for monovalent ions.

According to Ujang (1996) this membrane material operates best when the transmembrane pressure is sufficiently high to generate fluxes that are greater than those normally acceptable in RO installations. Superior resistance to fouling appeared to permit the use of sulphonated polysulphone membranes at high flux.

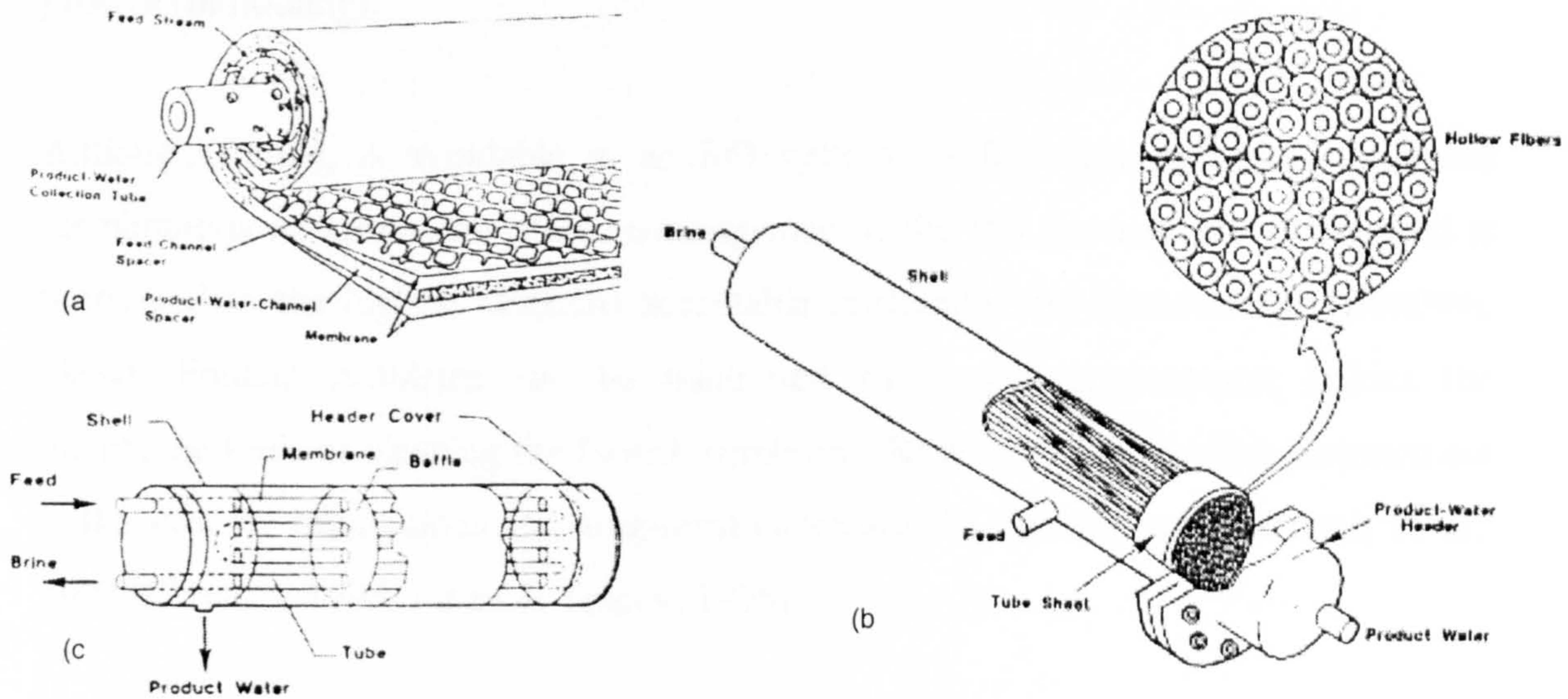


Figure 4.4 Membrane modules, (a) spiral wound, (b) hollow fibre and (c) tubular.
Source: Brandt et al., (1993)

4.5.4 Fouling problems

Fouling was defined by Gekas (1988) as the deposition of material on a membrane surface and/or in its pores, leading to a change in the membrane performance. He further explained that fouling was due to coupling of deposited layers caused by either foreign unwanted materials in a fluid or by the components to be retained. The process was due to concentration polarization. Fouling is considered a serious problem in membrane operations (Amjad, 1988; Fountoukidis et al., 1989; Kronmiller, 1994) resulting in flux decrease, shutdown of operation, replacement of membrane, etc.

Amjad (1988) further considered that there were two types of foulants in RO operation, namely mineral scales which are hard, dense, and crystalline precipitates and deposits such as colloidal and suspended matter, biological growth and silica. Others (Ebrahim, 1994; Finan & Tracey, 1995; Bhattacharyya et al., 1992) divided foulants

into: (i) suspended solids and particulates, (ii) colloids, (iii) scale forming salts, (iv) metal oxides, (v) biological foulants, and (vi) organic foulants. Brandt et al., (1993) also included oil and grease, chlorine and other oxidants. Flemming (1993) classified the cause of fouling as inorganic deposits (scaling), organic molecule adsorption (organic fouling), particulate deposition (colloidal fouling), and microbial adhesion and growth (biofouling).

Although fouling is avoidable in an RO system, in full-scale operations a fouled membrane is an almost inevitable consequence of the RO process, unless the feed is pretreated to the highest standard acceptable practically and economically (Ebrahim, 1994). Fouling problems can be minimised by proper pretreatment before the membrane fouls or cleaning the fouled membrane. Researchers agree that pretreatment is the key, or foundations for long-term successful RO performance (Brandt et al., 1993; Ebrahim, 1994; Finan & Tracey, 1995).

To minimise scaling or fouling, a study of the types of foulants is crucial. The design of an RO membrane system should involve an evaluation of the feed (Hooley et al., 1993). This is aimed at recognising any foulants which may affect the system. The presence of suspended solids in the feed tends to cause gross plugging of the device followed by fouling the membrane surface. Mineral scales usually consist of calcium carbonate, calcium sulphate, barium sulphate and calcium fluoride (Brandt et al., 1993; Amjad, 1988).

Membrane manufacturers generally specify a minimum requirement for colloidal or suspended materials using SDI (Silt Density Index) of the feed water. The SDI value is derived from the time required to filter a standard volume of water through a membrane at a constant pressure. Binovi and Kinman (1984) suggested the use of permanganate demand to replace SDI since the latter does not predict the lifespan of the membrane nor does it linearly react with concentration of potential foulants.

4.5.5 Pretreatment

Pretreatment is classified into two categories: (i) the prevention of chemical damage to the membrane and (ii) the prevention of fouling. As mention in § 4.5.3, some membranes are prone to adverse effect from chlorine, low pH, and high temperature. Chlorine is usually present if the feed water has been chlorinated as a control of microbial growth. Dechlorination may then be required. On the other hand, fouling usually results from foulants in the feed water (see § 4.5.4). Some recommended pretreatment methods are shown in Table 4.2.

Anti-scalants have also been introduced as pretreatment for RO. The compounds reduce the rate of scale formation so that the solution concentration remains above the solubility limit. Many compounds are now available, the most common one to control calcium sulphate formation being sodium hexametaphosphate or SHMP (Bhattacharyya et al. 1992b). Amjad (1985) studied the application of some anti-scalants to control calcium sulphate scaling. His study was based on crystal formation in a supersaturated calcium sulphate solution. He found that in the presence of an anti-scalant the crystallization was preceeded by an induction period. The induction period depended upon the anti-scalant concentration, the nature of the functional group, and the molecular weight of the compounds. Some compounds used as anti-scalant are formulated polyelectrolytes (e.g. AF-400), polyacrylates (at different molecular weight), polyphosphates and phosphonates (e.g. sodium pyrophosphate-SPP; sodium hexametaphosphate-SHMP; organophosphate such as HEDP), polystyrene sulphonates, and polyacrylamide (non-charged). The mechanisms of scale formation and inhibition were further studied by Amjad in 1988. Jaffer (1994) studied the use of acid together with an anti-scalant as a method to mitigate scaling and fouling.

Some pretreatment methods lead to further fouling problems as referred to by Ebrahim (1994). The use of phosphate anti-scalants, organic biocides, oxygen or oxidants, and activated carbon, may in some cases create organic slime or stimulate microbial growth, hence there is a need to have a proper or programmed cleaning of the membrane.

4.5.6 Membrane cleaning

To obtain successful membrane cleaning, the type of foulant should first be identified. The best way to do this is by a membrane autopsy which is very expensive. Other ways to identify it is by chemical and biological analyses of the feed water; analysis of acid, caustic and distilled water extracts of the prefilter, and using 0.45 micron Millipore filter to collect inorganic foulants which should be digested in acid or base before X-ray dispersive analysis (Ebrahim, 1994).

There are 3 types of membrane cleaning, namely: chemical, physical, and physico-chemical methods. Manufacturers usually recommend a specific membrane cleaning procedure for their products. Physical cleaning depends on mechanical treatment to dislodge the foulant from the membrane surface. Some of these are flushing (forward or reverse), permeate back pressure, vibration, air drain and water refill, air sparging (for hollow fibre), CO₂ back permeation (for hollow fibre), and automatic sponge ball (for a tubular module only). Physico-chemical cleaning uses physical methods along with chemical additions. This method, however, is not widely used in RO industry.

Chemical cleaning includes the use of acid or low pH solution, detergents, and commercial cleaning agents. Sulphate reducing bacteria have been successfully eliminated by paracetic acid and hydrogen peroxide at pH 2. Two percent EDTA and citric acid stabilised by ammonia to pH 7 was found to be good for CaSO₄ and CaCO₃ dissolution. Agents such as Flocclean have been reported to clean some foulants (Flocclean 511 at high pH suitable for biofouled membrane; Flocclean 411 to remove organics, silt and particulate from polyamide, polysulphone, and thin film composites, etc.). Detergents such as Biz and Ultrasil seemed to be effective for removing biofoulants (Ebrahim, 1994).

Table 4.2 Pretreatment methods used in desalination

Species	Problems	Pretreatment method	References
Suspended solids	Membrane fouling by particles causes reduced flux	(a) Sand filtration (b) Multimedia filtration (c) Coagulation filtration (d) Catridge filtration (e) Ultrafiltration	Bhattacharyya et al. (1992b)
Scale formations: (1). Ca/Mg bicarbonate (2) Ca sulphate scale (3) Silica scale (4) Iron precipitation	Membrane fouling by precipitates or scale causes reduced flux	(a) Base exchange softening (b) Lime softening (c) Acid dose (a) Base exchange (b) Add sequestrant (a) Raise temperature (b) Lime softening (a) Oxidation or aeration and filter (b) Exclude oxidation agent, e.g. air or Cl ₂ (c) Operate at low recovery (solubility not exceeded) (d) Chelating addition (e) Anti-scalant addition (f) Sand filtration to remove SiO ₂	Brandt et al. (1993) Bhattacharyya et al., (1992a; 1992b)
Colloids	Membrane fouling by colloids causes reduced flux	(a) Coagulation and filter (b) Base exchange softening (c) Ultrafiltration	Brandt et al. (1993) Bhattacharyya et al. (1992b)
Microorganisms	Slime layers on membrane causes reduced flux; some membrane degrades by microorganisms	(a) Chlorination (b) Sodium bisulphite addition (c) UV light (d) Ozonation (e) Copper sulphate addition (f) Chloramine	Brandt et al. (1993) Bhattacharyya et al. (1992a; 1992b) Flemming (1993)
Chlorine	Chlorine added as disinfectant will damage membrane	(a) Sodium bisulphite addition (b) Activated carbon filter	Brandt et al. (1993) Bhattacharyya et al. (1992b)
Organics	Adsorption on membrane can cause loss of water flux over time; some high MW organics can coagulate to form colloids	(a) Activated carbon (b) Replace use of cationic polymers (coagulant) which cause colloids formation	Bhattacharyya et al. (1992b)
Dissolved oxygen	Oxygen can damage some types of membrane; oxygen can increase corrosion problems	(a) Sodium bisulphite addition (b) Vacuum de-aeration	Bhattacharyya et al. (1992b)
pH	Should be in acceptable operation range of membrane	Adjusting with acid (HCl, H ₂ SO ₄) or base (lime, NaOH)	Bhattacharyya et al. (1992b)
Hydrogen sulphide	Not removed by membrane	(a) Oxidation (b) Air stripping (c) De-gas and Cl ₂	Bhattacharyya et al. (1992b) Brandt et al. (1993)

CHAPTER 5

AIMS OF STUDY

The study involved a contaminated land area with a history of industrial activity for over a hundred years. Various types of industries on the site have resulted in a cocktail of contaminants in the soil. This made the problems more difficult to deal with due to its heterogeneity. According to a preliminary study by consultants, however, the main problems in the contaminated area were related to the high sulphate level (see §3.2). In this study, the sulphate, some heavy metals (zinc, manganese, and copper) and arsenic would be the main contaminants of interest. Due to the complex nature of the area, the study only covered limited conditions and depended on the soil samples received. The samples studied were selected on the basis of depth and sulphate concentration.

The problems of the contaminated land studied were to be considered from two major sources. The first was the contaminated ground or soil and the second was the leachate generated from the area. Both have been observed to contain a high sulphate concentration and some heavy metals. The mixture of these contaminants in the soil are an unusual situation and have not been extensively studied in the area of contaminated land remediation.

The remediation of contaminated soil would be by the bioremediation alternative since it potentially offered a more economical and practical method for the deeply contaminated area. Sulphate was theoretically possible to be reduced biologically by either assimilatory or dissimilatory sulphate reduction processes (Paul & Clark, 1996; Killham, 1994). Heavy metals on the other hand could be separated as metal sulphide through microbial metabolism (Smith et. al., 1994). However, the contaminated soil studied was very much affected by the composition of the wastes dumped in the area and the physical and biochemical processes over a hundred years. The first aim of the study therefore, was to investigate the applicability of bioremediation to the soil studied by enhancing the microbial activity in the soil.

The soil composition and the biochemical reactions occurring in soil together with the existing hydrogeological processes had a significant effect on the leachate quality. Remediation of the soil by engineering practices and chemicals' addition at certain depth would also have an effect on the soil condition and quality. In the long run, it was assumed that if the soil composition changed, the leachate quality would also be altered. At the present time, leachate was the most obvious problem to deal with since it discharges directly to the River Tyne. The leachate treatment proposed and agreed upon by the Gateshead Metropolitan Borough Council (GMBC), was the application of reverse osmosis to reduce the contaminants discharged to the River. Consequently, this formed the basis for the second aim of the study.

To assess the application of bioremediation processes for the contaminated soil studied, three main factors were considered as follows: (see § 2.3.3)

- i). documented loss of contaminants from the site,
- ii). laboratory assays showing that the microorganisms were able to grow on the site samples, and
- iii). evidence of the biodegradation potential by detecting a change in reactants and products that may be indicative of known metabolic processes (Madsen, 1991; MacDonald & Rittmann, 1993).

These factors together with a screening protocol for investigating the remediation technique (see § 2.3.4) resulted in the objectives of a bioremediation study that included:

- i). investigation of the response of the soil contaminants to nutrient, pH, and carbon source amendments in the slurry phase,
- ii). investigation of microbial growth in the adjusted environment, and
- iii). investigation of the behaviour of contaminants in the soil column.

Similarly, the application of reverse osmosis to the treatment of leachate was studied. Since membrane life and fouling characteristics are of primary importance in a practical application, the objectives of leachate treatment by reverse osmosis were:

- i). study of fouling the reverse osmosis membrane when fed by artificial leachate as a preliminary investigation on membrane capability, and
- ii). determination of leachate pre-treatment process to minimise the fouling problems.

The whole study was carried out in stages, depending on the requirement or assessment of the bioremediation and reverse osmosis applications. More details of the objectives in each stage are considered and discussed in related chapters.

CHAPTER 6

MATERIALS AND METHODS

6.1 Description of Experimental Equipment and Methods

6.1.1 Slurry test

A slurry reactor was used to investigate the treatability of soil by biological processes. The soil slurry was made of a 1:10 proportion ratio (w/v) soil sample:water mixture. The soil samples used for the experiment were prepared as air-dried soil passing 2mm mesh sieve. The soil was stored at room temperature in double polythene bags which were sprinkled with water between the plastic bag to maintain its moisture content and to minimise evaporation (William and Gray, 1973).

The study involved 3 types of reactor for 5 soil samples. The first method used a 1 litre round flat bottom flask at room temperature for aerobic conditions. The second used an adaptation of the biometer, used by Bartha & Pramer (1965), placed in an orbital shaker and waterbath at 36-37° Celcius for the anaerobic conditions. The third method employed a manometric respirometer to measure more accurate gas evolution from the soil slurry for both aerobic and anaerobic conditions. The equipment arrangement is described in Figures 6.1, 6.2, and 6.3. Soil samples 1-3 were used in aerobic and anaerobic slurried soil tests, whereas Samples 4 and 5 were used in slurried soil with respirometer tests.

The study only covered five soil samples that were collected by the consultant at different depth and different sulphate concentration. Soil samples 1-3 were obtained in the same time at the beginning of the study period and soil samples 4 and 5 were received later. The structure of soil samples 1 and 2 were stiff friable grey/dark grey compressed silt. Soil sample 3 which was considered uncontaminated was soft dark brown very silty clay with some root material available. Soil 4 was grey very sandy silt containing fine to coarse gravel. Soil 5 was firm yellowy grey and dark grey silt. The soil samples' characteristics are illustrated in Table 6.1.

Table 6.1 Soil samples characteristics (in mg/kg dried soil, unless stated & pH) .

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Location	11m depth	9 m depth	surface	-	4m depth
PH	8.39	8.28	7.53	12.39	7.92
Moisture (%)	15.4	17.7	17.7	9.5	1.2
LOI (%)	9.4	9.6	13.7	13.2	8.5
Organic nitrogen	490	410	795	1025	450
SO ₄ ⁼	5610	5490	290	8940	4760
Ca	43900	44000	2800	50910	39710
Mg	4680	3780	4480	6490	5790
Na	1740	2700	300	5140	3170
K	320	480	980	760	1300
Zn	1780	1320	770	1290	2740
Pb	650	430	240	495	620
Mn	1330	1570	1010	950	860
Cu	210	260	100	270	140
As	240	240	370	270	160
HPC (cells/g)	2.59.10 ⁵	1.84.10 ⁵	1.65.10 ⁵	ND	1.56.10 ⁵
Total Count (cells/g)	NA	NA	NA	4.43.10 ⁵	5.37.10 ⁶

Note: ND= not detected
 NA= not analysed
 LOI=Loss on ignition
 HPC=Heterotrophic plate count

The study investigated the reaction of the soil slurry with different nutrient supply and different environments. Tables 6.1, 6.2 and 6.3 describe the experimental programme for each type of slurry test.

The main analyses were:

- i). carbon dioxide evolution from the reaction to assess the microbial activity assessment, and
- ii). chemical analysis of pH, sulphate, and those heavy metals of interest.

The volatile suspended solids content was also measured in the aerobic 1 litre flask slurry test.

The nutrient added to the flasks in aerobic slurried soil tests was a mixture of NH₄HCO₃ (N), KH₂PO₄ (P1) and K₂HPO₄ (P2) as N and P sources. The maximum concentration was approximately 10mg/l, 5mg/l and 6mg/l of reactor volume respectively (Irvine et.al., 1993). Each nutrient was added at 3 concentration levels, namely 25%, 50% and 100% of the maximum nutrient concentration.

In both anearobic and respirometer slurried soil tests the effect of other types of nutrient namely urea as the N only source and Omex as the N+P+trace elements source were also investigated. In respirometer tests there were 4 types of nutrient added to the flasks:

- i). N+P source using similar nutrients as in aerobic method,
- ii). N only source which use urea as in anaerobic method,
- iii). P only source using KH_2PO_4 and K_2HPO_4 in a similar concentration to aerobic method, and
- iv). N+P+trace elements using Omex as in anaerobic method.

Omex is a concentrated commercial nutrient available which contains a source of nitrogen, phosphorous and other elements. The specific one used in this study was a product with nitrogen, phosphorous, and trace elements. The concentration applied was 1ml in 10 litres (McDougall, 1996).

In respirometer test (Method III) nutrients were added at 3 concentration levels. The effect furthermore, of glucose as an additional carbon source, and pH adjustment in the soil samples were also investigated.

i. Aerobic condition (Method I)

The reactor used for investigating the reaction of soil under aerobic conditions used a 1 litre round flat bottom with 2 side inlets for pH measurement and sample collection (see Figure 6.1). The flask was placed in a stirrer and supplied by air from a small aquarium pump. The air flow was maintained at 1.8-2 l/min prior to agitation. Gases from the headspace of the flask were connected to an alkali container as a CO_2 trap. The alkali was titrated with acid at the end of the agitation to measure the CO_2 evolution from the system. Water loss due to evaporation was replaced by distilled water (Irvine et al., 1993)

The soil slurry was made of 50g air-dried soil: 500ml overnight tap water (collected tap water was left overnight before use) mixture which was aerated and agitated with a magnetic stirrer. Supernatants were sampled after 22, 46 and 70 hours for pH, volatile suspended solids content, sulphate and zinc measurement. The microbial activity was

measured by the production of CO_2 using NaOH which reacted with the CO_2 produced from the slurry reaction. Titration as a method to estimate respiration was considered to be more robust than using other respiration measurement (Cleve et al., 1979). The slurry test was operated according to a sequencing batch reactor system (Irvine et. al., 1993) which comprised fill, react, settle, and draw phases. The experiment was carried out at room temperature. This aerobic method examined soil samples 1, 2, and 3.

There were four treatment options tested for each sample. Three treatments used aeration and one was without aeration. The first flask was only aerated, the second flask was supplied with nutrient and aeration, in the third flask activated sludge was also added along with nutrient and aeration whereas the fourth was only supplied with activated sludge without aeration. One set of experiments for each sample comprised of:

- i). 'once nutrient supplied' and samplings were carried out approximately at 22, 46, or 76 hr. intervals, and
- ii). 'daily nutrient supplied' of three level of nutrient concentration (25%, 50% and 100% of maximum nutrient concentration used) with similar sampling intervals.

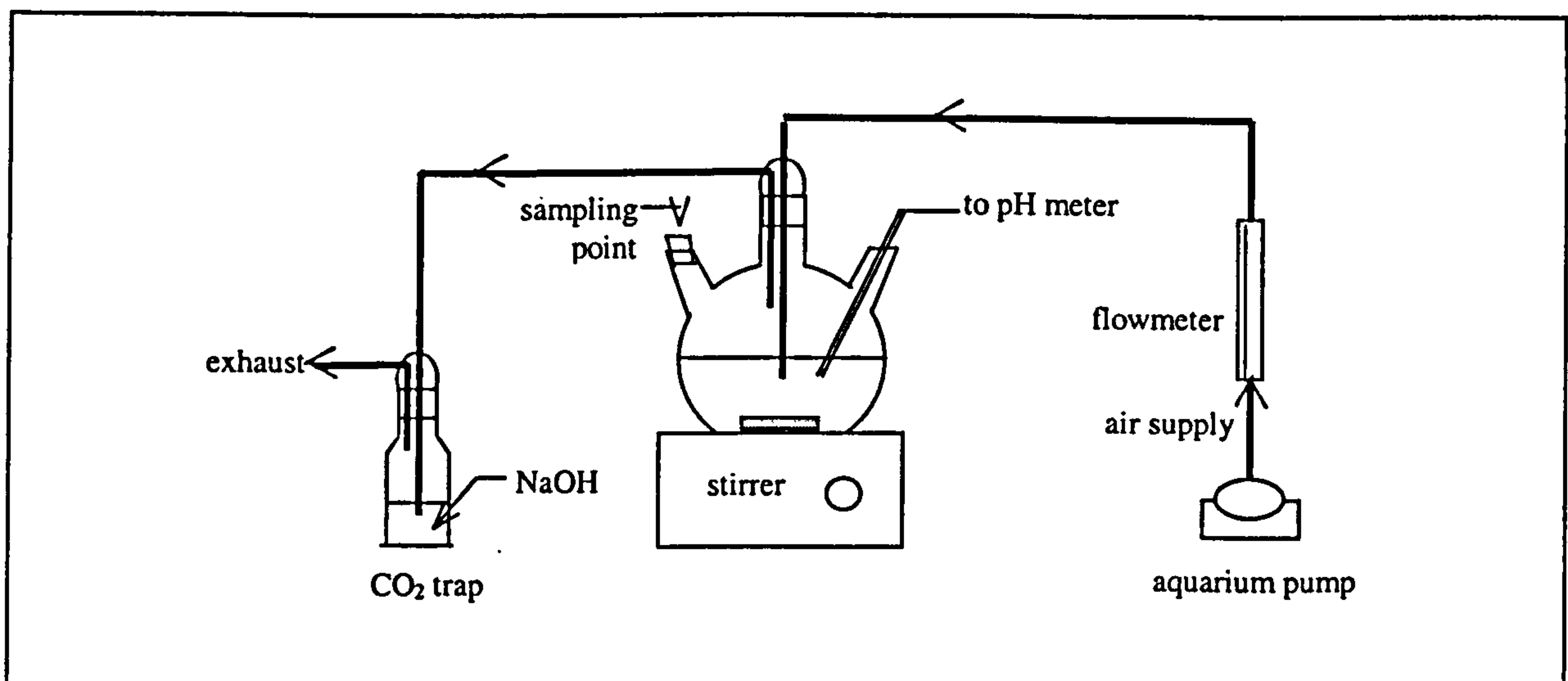


Figure 6.1 Arrangement of aerobic soil slurry test equipment (Method I)

ii. Anaerobic condition (Method II)

The flask used for the experiment was adopted from the Bellco flask used by Bartha and Pramer (1965) to measure the persistence and biological effects of pesticides in soil. Flasks were set up to determine biodegradation in soil by measuring CO₂ evolution as reported by Sharabi and Bartha (1993). This study used the flask to investigate slurried soil in anaerobic condition.

The biometer flask was a 250ml Erlenmeyer sealed with rubber a stopper and equipped with a stopcock in which filled with carbosorb as carbon dioxide adsorber (see Figure 6.2). The carbosorb was used to prevent any CO₂ from air and injected gas such as nitrogen. The flask had a rubber tubing extension to allow connection to a nitrogen gas source to maintain anaerobic condition in the flask. A 50ml side tube was fused to the flask as an alkali trap to collect CO₂ produced from the slurry. The tube was covered by a self-sealed septum into which a syringe needle of 12cm was inserted to deliver and withdraw the alkali. The tip of the needle was covered with polyethylene tubing to make complete withdrawal of alkali possible. During the operation, the needle was always capped by its cover. The alkali used, 1N NaOH was titrated using 1N H₂SO₄ for each sample to measure CO₂ evolution from the system. The alkali was transferred out from the side tube by syringe connected into the needle. To collect all the alkali for titration, the side tube was washed out by injecting deionised water. The rinse water was then collected for titration.

A soil slurry of 10g dried soil: 100ml overnight tap water mixture was agitated for a similar period as in aerobic procedure (Method I). In the first treatment option, the flasks were agitated in an orbital shaker at 80rpm at room temperature. Nutrient was added in similar proportions to those in Method I. Anaerobic condition were maintained in the headspace of the flask. The experiment was started after flushing the flasks with nitrogen gas for 3-5 minutes to initiate the anaerobic condition. In treatment options 2 and 3 the reaction was agitated in a water bath at 36-37°C. Each soil sample was tested in duplicate flasks.

iii. Aerobic and anaerobic conditions (Method III)

The respirometer used for the experiment was a Warburg apparatus, operated manually. The flask attached to the manometer was designed to accommodate the gas sampling collection. The volume of the flask was around 50ml without the centre-well containing the alkali. The flask was fused with a side arm which was covered by self-sealed septum. This made it possible to inject the material under investigation and also to collect gas at the end of the operation. Seven flasks were used in each run. Six flasks were filled with soil slurry and one flask with deionised water of a similar volume setting as the control allowing barometer changes.

Gas evolution is calculated using $x = h.K$ where h is the change in the reading in the open arm of the manometer (in unit) and K is the flask constant. The flask constant is calculated using the following equation (6.1)

$$K = \frac{V_g \frac{273}{T} + V_f \alpha}{P_0}$$

where V_g = The volume of gas phase in the flask including the connecting tube down to the reference point in the manometer (μ l),

V_f = The volume of liquid in the reaction flask (μ l),

P_0 = Standard pressure of Brodies fluid (10,000mm)

T = Temperature in the waterbath (273 + degree in Centigrade)

α = Solubility in the reaction liquid of the gas evolved (0.028)

The slurry was made up using 1 g dried soil sample: 10ml distilled water. Prior to any addition of nutrient or other material, the soil slurry was homogenized by operating the Warburg for 6 hours continuously. In trial experiment, it was noted that gas evolution from slurried soil was fluctuated up to 320 minutes shaking. The material studied, such as nutrient or glucose, were injected after homogenization. The Warburg was operated for a period of approximately 24 hours at 25°C and 50rpm. Readings from the open end of the manometers in Warburg respirometer were carried out over 3 hours every 10 minutes in the first half an hour, 3 times 20 minutes, and 3 times 30

minutes. Samples of gas and supernatant were taken after 24 hours total operation. Gas samples were analysed using a gas chromatograph Becker model 403 with Unicam 4815 integrator.

Aerobic or anaerobic conditions were maintained in the headspace of the flask. In the anaerobic system, the flasks were flushed using nitrogen gas free of oxygen just before the start of each experiment. The slurry had been through the soil homogenization procedure prior to any material addition. The anaerobic system was also run a similar way to the aerobic system.

In the acidified soil sample, the soil mixtures were acidified using hydrochloric acid. The acidification was carried out to obtain a soil mixture at neutral pH (around 7). The acidification procedure of the soil mixture was completed when gas generated from the reaction was considered to be steady. Acidification of the soil mixture was carried out in a biometer flask (see Appendix C).

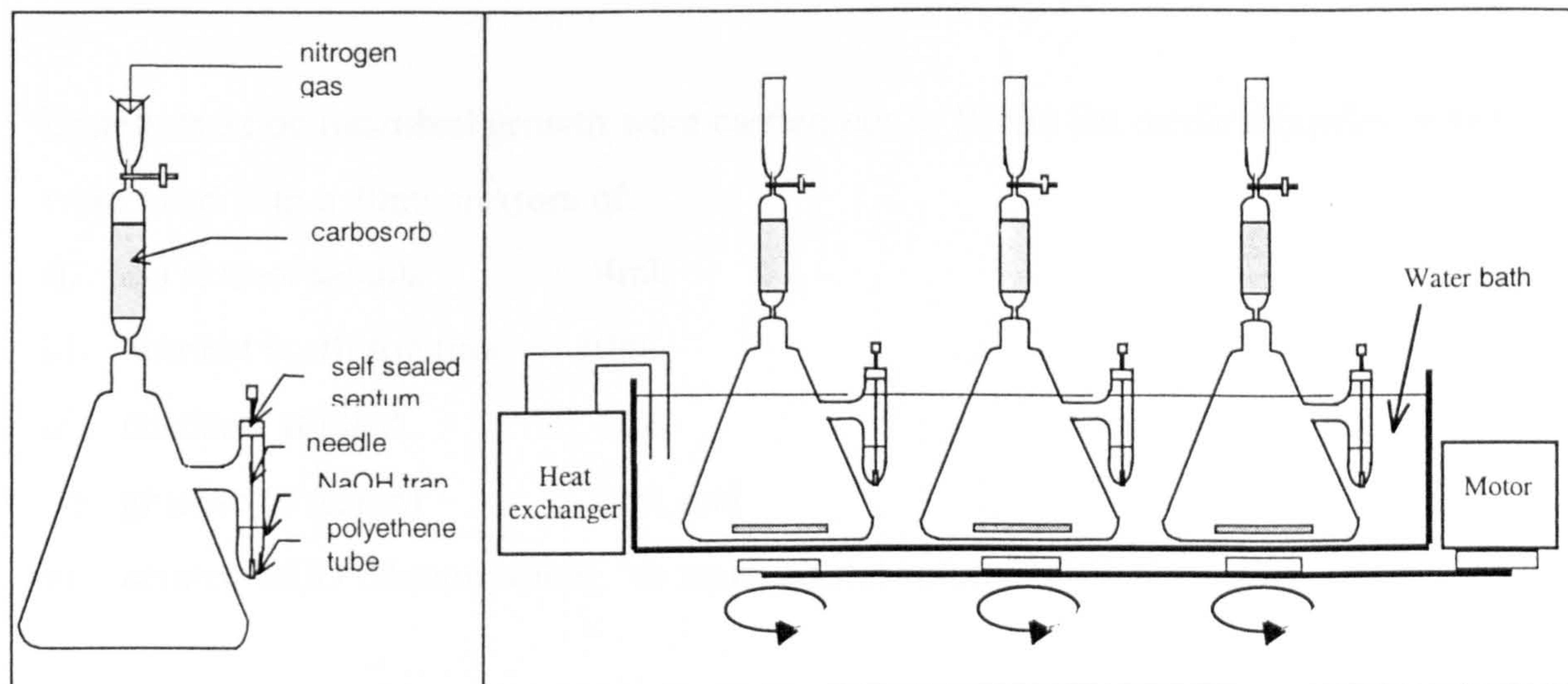


Figure 6.2 Arrangement of anaerobic soil slurry test equipment (Method II)

6.1.2 Microbial growth assessment

The turbidity of a liquid medium has been used to assess microbial growth in microbiological research for decades. This principle was adopted by Alsop et al. (1980) who investigated the toxicity of materials to microbial cultures. McClure et al. (1993)

used an automated turbidimeter to construct a kinetic growth model of *Listeria monocytogenes*. Alsop et al. (1980) claimed that microbial growth as an assessment of microbial activity offered a lower equipment cost requirement and is more sensitive than respiration measurements.

In this study, the microbial growth test of the soil extract was a modification of the method proposed by Alsop et al. (1980). The procedures and equipment used were tested prior to the study. These included releasing microbes from soil particles, soil and dilution solution mixture, the bottle used and the period of shaking (see § 7.3).

The soil extract was obtained from a mixture of air-dried sieved soil and distilled water at a ration of 1:10 (5g soil: 50ml distilled water) in a 250ml Erlenmeyer flask covered with cotton wool. The mixture was shaken in an electrical orbital shaker for 16 hours at 25°C. Soil extract for the experiment was collected after filtering through a Whatmann No. 1 filter paper. Bakken (1985) used distilled water to separate microbes from soil particles following evaluation of the use of a range of dilution media.

Experiments on microbial growth were carried out in 100ml flat medical bottles which were filled with a 40ml mixture of:

- i). soil extract sample : 4ml,
- ii). nutrient broth solution : 10ml,
- iii). nutrients studied : 4ml,
- iv). glucose (if tested) : 1ml, and
- v). aerated BOD dilution water: to make a total volume of 40ml.

The samples were shaken in an electrical orbital shaker for 16 hours at 150rpm and at 25°C. The turbidity of the mixture was then measured as absorbance in spectrophotometer at a wavelength of 530nm. Some samples were studied at half the volume required due to limited soil sample extract.

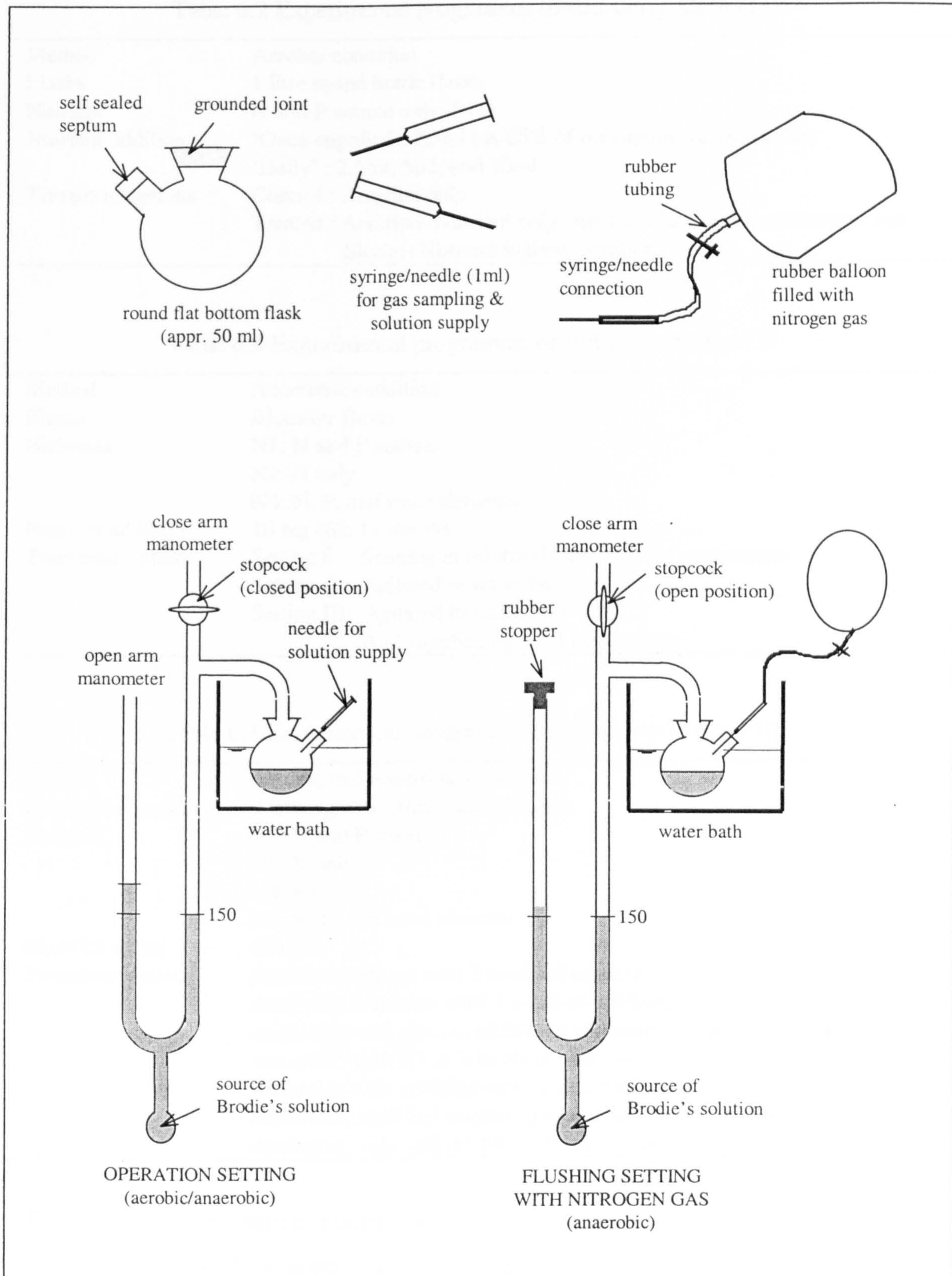


Figure 6.3 Arrangement of soil slurry test using respirometer (Method III)

Table 6.2 Experimental programme of soil slurry Method I

Method	Aerobic condition
Flasks	1 litre round bottle flasks
Nutrient	N and P source only (N1)
Nutrient addition	‘Once-supplied’: 2.5ml (as 25% of maximum concentration); ‘Daily’ : 2.5ml; 5ml; and 10ml
Treatment options	Control : Aeration only Treated : Aeration+Nutrient only; Aeration+act. Sludge+Nutrient; Act. Sludge+Nutrient without aeration

Table 6.3 Experimental programme of soil slurry Method II

Method	Anaerobic condition
Flasks	Biometer flasks
Nutrients	N1: N and P source N2: N only N4: N, P, and trace elements
Nutrient addition	10 mg N/l; 11 mg P/l
Treatment options	Setting I : Shaking in orbital shaker at room temperature Setting II : Agitated in water bath (36°C) Setting III: Agitated in water bath (36°C) And supplied by SRB populations

Table 6.4 Experimental programme of soil slurry Method III

Method	Aerobic and anaerobic conditions
Flasks & apparatus	Warburg with 50ml modified flask
Nutrient	N1: N and P source N2: N only N3: P only N4: N, P, and trace elements
Material added	Glucose
Treatment options	Aerobic condition with 3 levels of nutrient Anaerobic condition with 3 levels of nutrients Anaerobic with glucose addition and nutrients at medium level Anaerobic with N1 at 3 levels of glucose concentrations Anaerobic with acidified sample and nutrient at medium level Anaerobic, acidified sample, glucose and nutrient at medium level Anaerobic, acidified sample with N1 at 3 levels of glucose concentration

The microbial population number was estimated from the correlation between absorbance level and the direct total count of the mixture. The equation from the study using Microsoft Excel Program was

$$y = 9.10^6 \times e^{6.9064 \cdot x}$$
$$R^2 = 0.894$$

(6.2)

where: y = estimation of microbial number (cells/ml)
 x = absorbance of mixture at 530nm.

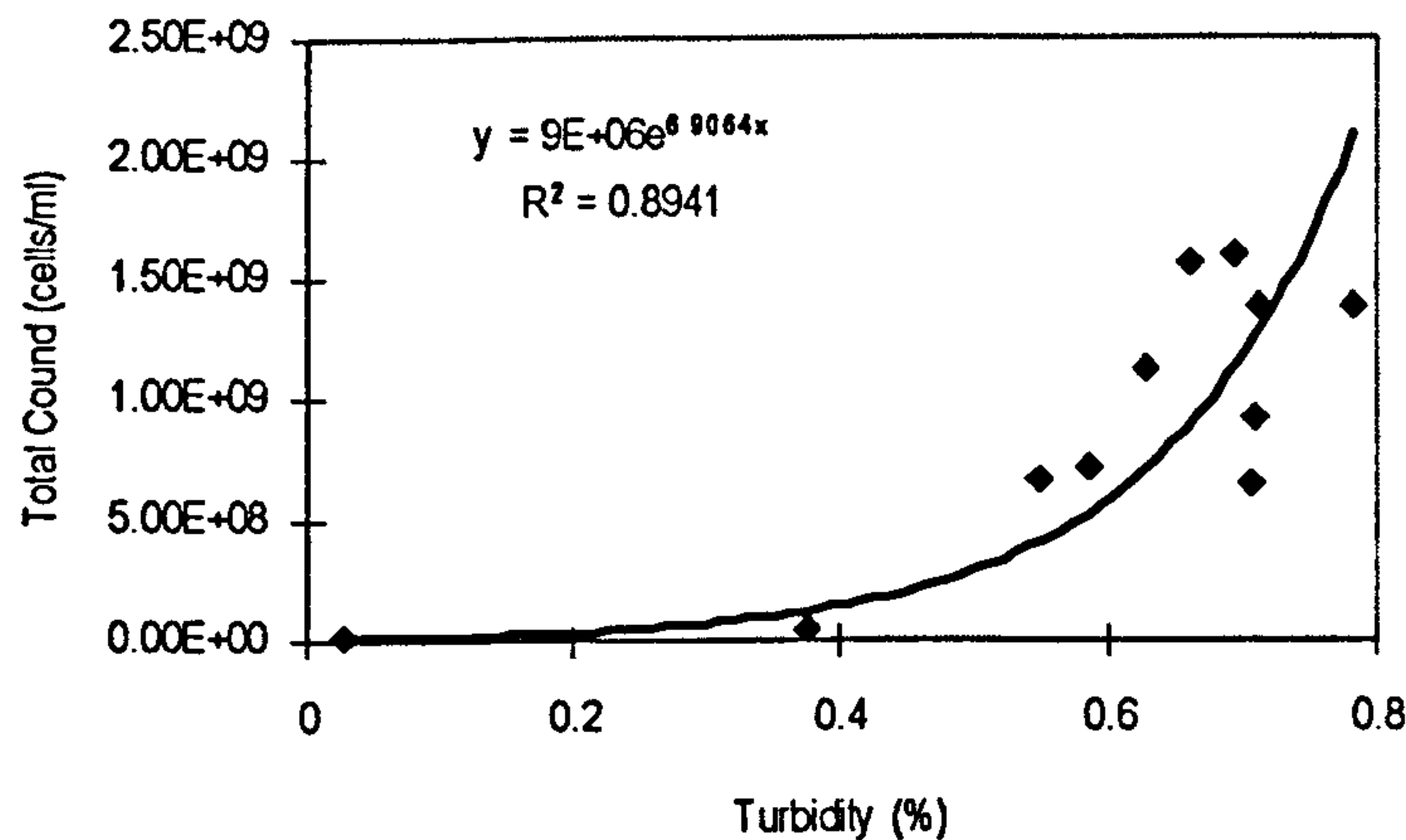


Figure 6.4 Relationship between absorbance and total count

6.1.3 Column test

A soil column test as the second stage of the treatability study was carried out in a Perspex tube with a diameter of approximately 70mm and a height of 1000mm. The arrangement of the soil column setting is illustrated in Figure 6.5. The column was equipped with an air supply, a rotameter and a trap for CO₂ evolution. Air was supplied from a central air compressor after flowing through an alkali solution to trap CO₂ before it reacted with the soil. The air flow was maintained at 4 litre/min. for the two columns studied. The nutrient and glucose addition was from top of the column. The leachate generated was collected at the end of the column and was sampled every day. The column was also equipped with soil sampling points at 3 column heights at different angles. A soil sample was obtained using a small metal core to withdraw the soil. Soil samples from the column were collected at the end of each treatment.

The analyses carried out during soil column tests included:

- i) carbon dioxide evolution,
- ii) pH, sulphate and heavy metal concentrations in leachate,
- iii) hydrogen sulphide gas from the column,

- iv) pH, sulphate, and heavy metal concentrations in soil samples,
- v) microbial numbers in soil samples, and
- vi) COD and volatile fatty acids in the leachate collected after addition of glucose.

The column was filled with prepared soil samples 4 and 5 (air-dried and sieved). In the study 4 types of treatment or material addition to the column was investigated. These include deionised water supply, deionised water and air, nutrient solution, and glucose and nutrient. The nutrient added to the column was that which gave the highest respiration results in slurry test using respirometer (Method III). The nutrient was added daily and continued for approximately 3-4 weeks for each treatment.

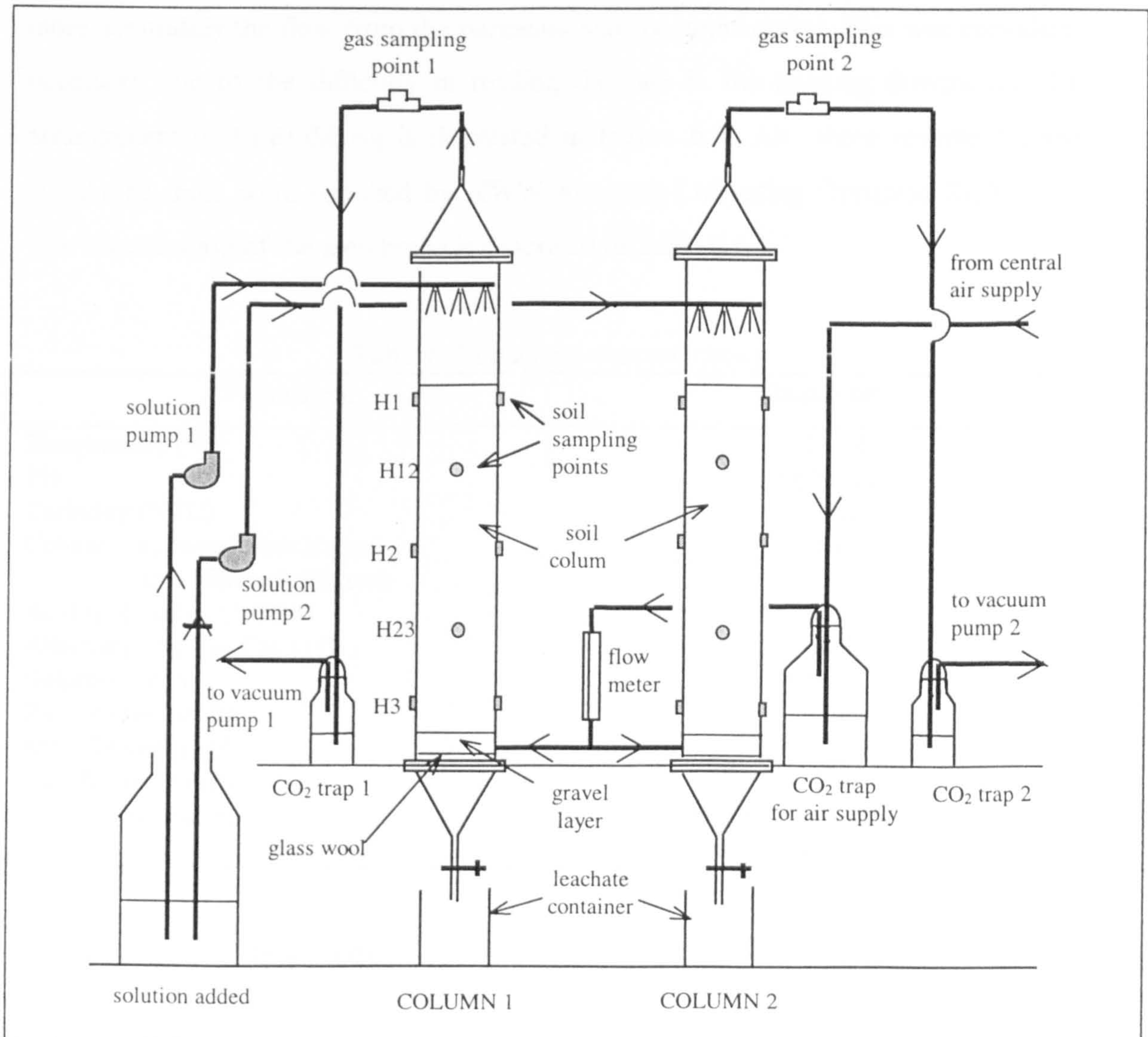


Figure 6.5 Arrangement of soil column reactor

A pull-out explanatory note of conditions and settings in bioremediation studies is available after page 176.

6.1.4 Reverse osmosis study

The study of leachate treatment employed a low pressure reverse osmosis membrane (LPROM). The leachate characteristic studied is illustrated in Table 6.5. The reverse osmosis rig was designed and used by Ujang (1996) to investigate the application of a LPROM to remove heavy metals from industrial wastes. The material used for construction of the rig was selected from high quality plastics and stainless steel to prevent contamination and leaking of pressurised flow of the feed. An additional accessory to the previous application was the addition of two burettes to measure more accurately the flow from the permeate and the concentrate. This was considered necessary due to the difficulty in reading the rate in the existing flowmeters. The arrangement of LPROM rig is illustrated in Figure 6.7. All three reverse osmosis membrane units were supplied by NWW-Acumem Ltd. using Optimem R02012-16. The specification of the membrane is described in Table 6.6.

Table 6.5 Leachate characteristics

Parameters	Concentration
Temperature (°C)	13-14
PH	7.62 - 8.04
Turbidity (NTU)	350
Colour: apparent (unit Hazen)	500
true (filtered) (Hazen)	30
Acidity (mg/l as CaCO3)	32
Alkalinity (mg/l as CaCO3)	493
Sulphate (mg/l)	3290 - 3750
Zn - filtered (mg/l)	0.062
Mn - filtered (mg/l)	0.148
Na - filtered (mg/l)	730
Physical appearance	white & cloudy but changing with time and storage

i. Membrane fouling study

The study was aimed at investigating the effect of sulphate concentration and applied pressure on membrane fouling (2 factors). The temperature in all runs was maintained

at 30°C. Each factor as variables were studied at five levels of conditions as shown in Table 6.5.

Table 6.6 Specification of LPROM unit

Membrane material	: Sulphonated polysulphone
Membrane configuration	: Spiral wound
Mode of operation	: Continuous
Dimensions	: Refer to Figure 6.6
Active surface area	: 0.465 m ²
pH range	: 2 - 11 for continuous operation 1 - 13 for short term exposure
Temperature	: 2 - 45°C
Pressure	: Maximum of 125 psi
Rejection at 414kPa, 25°C	: 95-97% for softened tap water 90-94% for hard tap water
Charge	: Negative
Thickness	: 150-175 micron (<1 micron active layer)
Maximum Silt density index	: 5 Silt Density Index
Maximum Feed water turbidity	: 1.0 NTU
Maximum TDS	: 2000 ppm
Maximum Feed flow rate	: 2 gpm
Expected life span	: 3 years
Special characteristics	: Chlorine resistance and low pressure operation
Manufacturer	: NWW-Acumem Ltd.
Product commercial code	: Optimem RO2012-16

Source: Ujang (1996)

Table 6.7 Experimental programme for membrane fouling study

Setting and codes	Operating pressures (kPa)	Sulphate concentration (mM)
Low-Low (LL)	375	10
Low (L)	425	11.25
Medium (M)	475	12.5
High (H)	525	13.75
High-High (HH)	575	15

The experiment was run according to factorial design. The first step used 2² factorial design i.e. two factor each at two levels. The second step used Star design as suggested by Ujang (1996). The complete experiment programme was as follows:

Table 6.8 2^2 factorial combination

Combinations	Pressure (kPa)	Concentration $\text{SO}_4^{=}$ (mM)
1	L	H
2	H	L
3	L	L
4	H	H

Table 6.9. Star design

Combinations	Pressure (kPa)	Concentration $\text{SO}_4^{=}$ (mM)
5	M	M
6	HH	M
7	LL	M
8	M	HH
9	M	LL

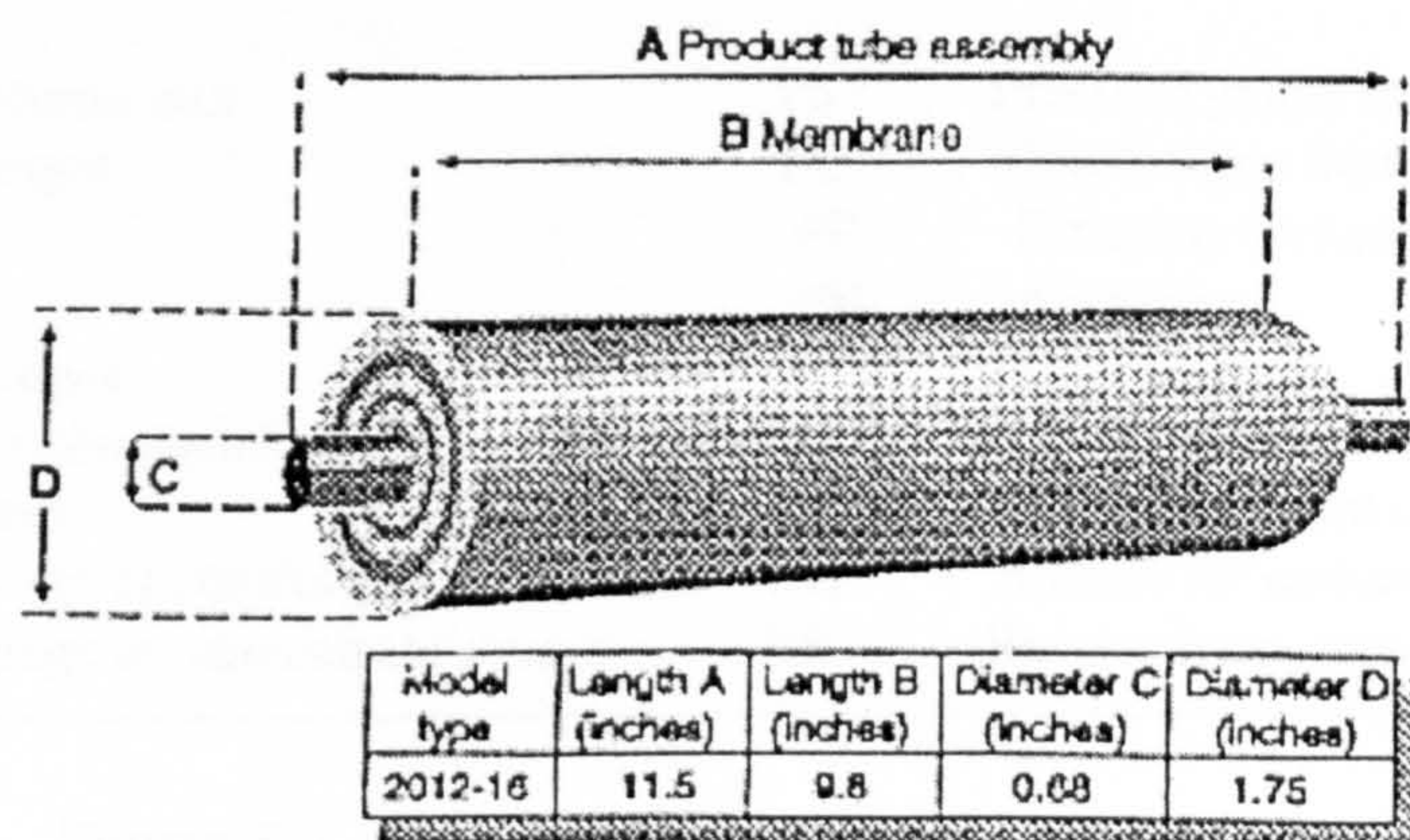


Figure 6.6 Dimensions of the RO Membrane

Source : NWW-Acumem Ltd.

Sampling

The sampling frequency was carried at intervals of 10 to 15 minutes at the beginning of each run and every 30 minutes thereafter. Sampling included:

- checking and adjusting pressures in P1, P2, and P3,
- measuring pH, temperature and conductivity in the feed tank and permeate,
- taking samples for sulphate, chloride and heavy metals analyses from permeate, and
- monitoring flow rates of concentrate and permeate.

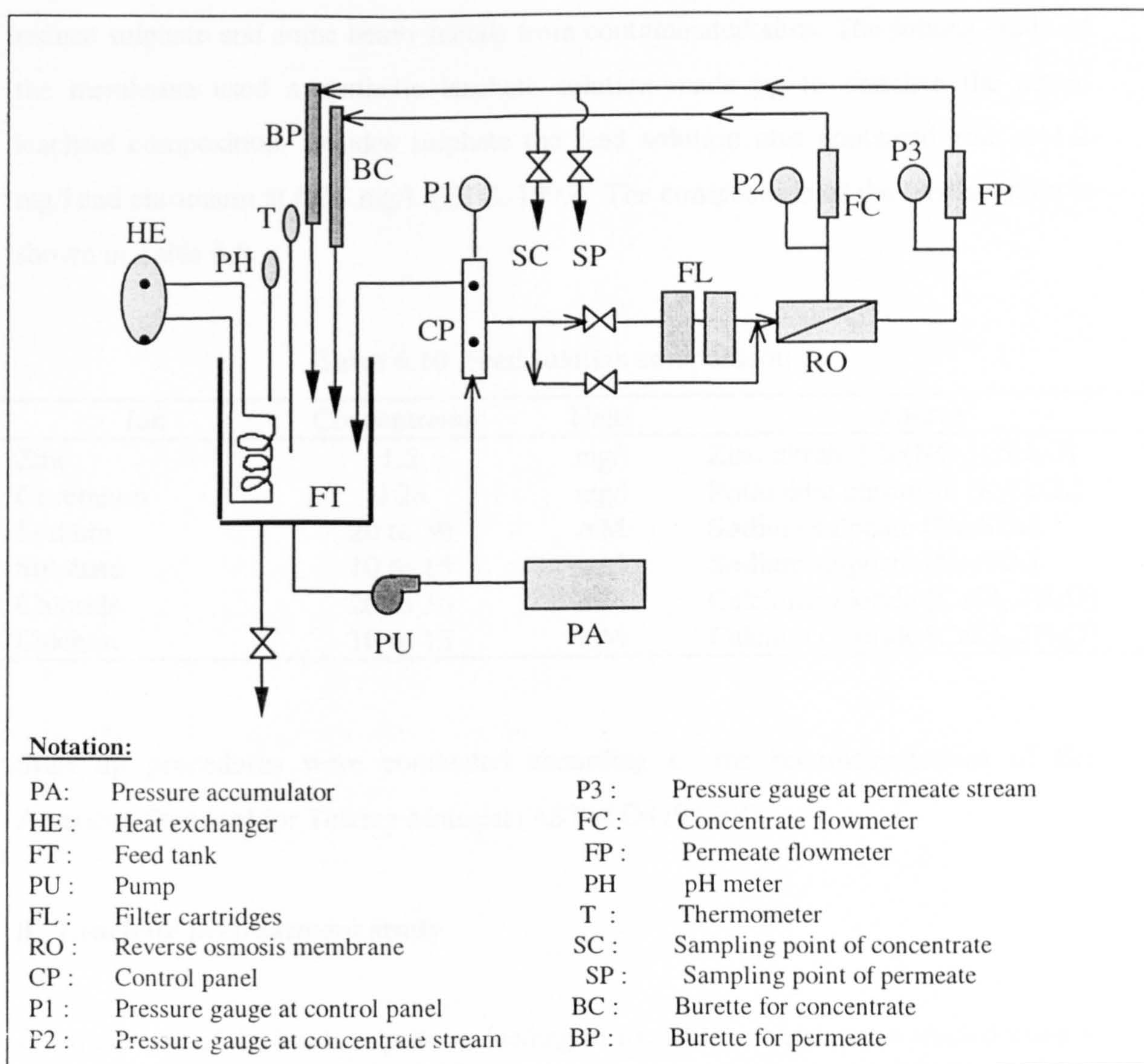


Figure 6.7. Low pressure reverse osmosis system

Pretreatment filters

The reverse osmosis rig was equipped with 2 filter cartridges as pre-treatment units. This was intended to minimise any effects from suspended matter in the feed solution. Two filters were used, both supplied by Memtec America Corporation. Smoker (1996) recommended the use of their Poly-Matrix™ Series cartridges which were polypropylene melt spun depth filters and claimed to be suitable for RO pre-treatment and for use with leachate. This study used 2 of these in series with micron ratings of 50 μ and 5 μ .

Feed solution

The study was carried out to investigate the use of the reverse osmosis membrane to

reduce sulphate and some heavy metals from contaminated sites. The fouling study of the membrane used a synthetic leachate solution made up to simulate the actual leachate composition. Besides sulphate the feed solution also contained zinc at 1.2 mg/l and chromium at 0.26 mg/l (ETC, 1995). The composition of the feed solution is shown in Table 6.8.

Table 6.10. Feed solution composition

Ion	Concentration	Units	Source
Zinc	1.2	mg/l	Zinc nitrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$]
Chromium	0.26	mg/l	Potassium chromate [K_2CrO_4]
Sodium	20 to 30	mM	Sodium sulphate [Na_2SO_4]
Sulphate	10 to 15	mM	Sodium sulphate [Na_2SO_4]
Chloride	20 to 30	mM	Calcium chloride [$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$]
Calcium	10 to 15	mM	Calcium chloride [$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$]

Start up procedures were conducted according to the recommendations of the American Standard for Testing Material (ASTM D4194-89).

ii. Leachate pretreatment study

Pre-treatment of the leachate prior to feeding to the RO membrane was studied using a coagulation-flocculation processes. Samples in the Jar Test apparatus were reacted with FeCl_3 , $\text{Al}_2(\text{SO}_4)_3$ as coagulants with the addition of a polyelectrolyte Zetag 92, and BaCl_2 5%. Zetag 92 was chosen according to the company profile and was intended for primary clarification with high cationic characteristic.

The first stage experiment was aimed at investigating the effects of adding coagulant, BaCl_2 , and Zetag 92. The second stage was intended to compare the performance which using ferric chloride or alum coagulants and BaCl_2 . The 2^3 factorial design of experiments was used in the first stage, and the two factorial design was applied for comparing the effects of coagulant types. Two levels of concentration were examined for each factor as illustrated follows:

Table 6.11 Concentrations used for coagulation test (mg/l)

Chemicals	Low	High
FeCl ₃ and Al ₂ (SO ₄) ₃	30	60
BaCl ₂	none	10
Zetag 92	none	10

For the second stage the concentration of ferric chloride, alum, and barium chloride was increased to 200 and 800 mg/l whereas the Zetag 92 concentration was maintained at a similar concentration (10 mg/l)

The analyses for the coagulation results included pH, turbidity, colour, sulphate concentration, volume of settled material and size of floc developed. The effect of pH adjustment to neutral prior to coagulation process was also investigated. The experiments were conducted in duplicate.

6.2 Design of experiments and statistical analysis

The design of experiments is a test (or series of tests) in which purposeful changes are made to the input variables of a process (or system) so that the reasons for changes in the output may be observed (identified), or to identify the sources of variability in the process (Montgomery, 1991). All the statistical analyses are carried out using the MINITAB release 9.2 programme.

The study was based on a fixed effect model, that is a model in which the conclusions will only apply to the factor level considered in the analysis. A factor is a collection of levels of a specific treatment. The statistical analysis involved simple comparative experiments, single-factor experiments and factorial design. Simple comparative considers a comparison of two conditions (or treatments), single-factor experiments further compare more levels of a factor and factorial design studies the effects of two or more factors.

6.2.1 Simple comparative experiment

Comparing two treatments or conditions statistically means to compare and make inferences concerning the difference between two populations means, μ_1 and μ_2 . There are two ways to compare two population i.e. using two sample t-test or paired t-test.

i. Two sample t-test

This was used to compare two independent populations with each μ_i (population means) and σ^2 (population variances) where $\sigma_1^2 = \sigma_2^2 = \sigma^2$. The hypotheses is

$$H_0: \mu_1 = \mu_2$$

$$H_1: \mu_1 \neq \mu_2$$

The test statistic t is given by:

$$t_o = \frac{\bar{y}_1 + \bar{y}_2}{S_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad (6.2)$$

where \bar{y}_1 and \bar{y}_2 are sample means, n_1 and n_2 are sample sizes.

S_p^2 is an estimate of the common variance $\sigma_1^2 = \sigma_2^2 = \sigma^2$ computed from:

$$S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2} \quad (6.3)$$

where S_1^2 and S_2^2 are the two individual sample variances.

Sample means is obtained from:

$$\bar{y} = \frac{\sum_{i=1}^n y_i}{n} \quad (6.4)$$

and sample variance is calculated from:

$$S^2 = \frac{\sum_{i=1}^n (y_i - \bar{y})^2}{n - 1} \quad (6.5)$$

The null hypothesis H_0 will be rejected if $|t_o| > t_{\alpha/2, n_1+n_2-2}$ for two sided rejection area with degree of freedom (DF) n_1+n_2-2 . The α is a significance level of probability to obtain error Type I which is rejected H_0 | H_0 is false.

In case in the assumption of $\sigma_1^2 \neq \sigma_2^2$, the test statistic is slightly different:

$$t_o = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}, \quad (6.6)$$

with degree of freedom:

$$v = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2} \right)}{\frac{\left(\frac{S_1^2}{n_1} \right)^2}{n_1 - 1} + \frac{\left(\frac{S_2^2}{n_2} \right)^2}{n_2 - 1}} \quad (6.7)$$

ii. Paired t-test

Some data are not independent but measured at successive times. For this type of data, the comparison of two means is obtained by matched pairs difference or paired t-test (Clarke, 1994). The difference of Treatment 1 and 2 is calculated for each pairs:

$$d_i = y_{1i} - y_{2i} \quad i = 1, 2, \dots, n \quad (6.8)$$

The hypothesis test:

$$H_0: \mu_d = 0$$

$$H_1: \mu_d \neq 0$$

The test statistic:

$$t_o = \frac{\bar{d}}{S_d \sqrt{n}} \quad (6.9)$$

where

$$\bar{d} = \frac{1}{n} \sum_{j=1}^n d_j \quad (6.10)$$

and

$$S_d = \left[\frac{\sum_{j=1}^n (d_j - \bar{d})^2}{n-1} \right]^{1/2}$$

$$= \left[\frac{\sum_{j=1}^n d_j^2 - \frac{1}{n} \left(\sum_{j=1}^n d_j \right)^2}{n-1} \right]^{1/2} \quad (6.11)$$

The null hypothesis is rejected if

$$|t_0| > t_{\alpha/2, n-1} \quad (\text{DF} = n - 1).$$

The confidence interval on $\mu_1 - \mu_2$ is

$$\bar{d} \pm t_{\alpha/2, n-1} S_d \sqrt{n}$$

With paired comparison there is a blocking principle which refers to a relatively homogeneous experimental unit. The proper use of blocking may reduce the variability and the confidence interval becomes narrower.

6.2.2 Single factor experiment

Another way to compare two conditions or treatments is by using a single-factor experiment with two levels of the factor. Furthermore, the single-factor experiment can also be used for more than two levels of the factor. The observation can be described as a linear statistical model:

$$y_{ij} = \mu + \tau_i + \epsilon_{ij} \quad \begin{array}{l} i = 1, 2, \dots, a \\ j = 1, 2, \dots, n \end{array} \quad (6.12)$$

where y_{ij} is the (ij) th observation, μ is an overall mean, τ_i is i th treatment effect, and ϵ_{ij} is a random error component. This model is called one way or single-factor analysis of variance. For the fixed effect model, the treatment effects τ_i are usually defined as deviations from the overall mean,

$$\sum_{i=1}^a \tau_i = 0$$

The hypotheses to test the equality of the a treatment means are:

$$H_0: \mu_1 = \mu_2 = \dots = \mu_a$$

$$H_1: \mu_i \neq \mu_j \quad \text{for at least one pair } (i, j)$$

Or in term of the treatment effects the hypotheses are:

$$H_0: \tau_1 = \tau_2 = \dots = \tau_a = 0$$

$$H_1: \tau_i \neq 0 \quad \text{for at least one } i$$

To test the equality of a treatment means, the analysis of variance is derived from a partitioning of total variability into its components part. The total corrected sum of the squares of the model is:

$$SS_T = SS_{Treatment} + SS_E$$

where

$$\begin{aligned} SS_T &= \sum_{i=1}^a \sum_{j=1}^n (y_{ij} - \bar{y}_{..})^2 \\ &= \sum_{i=1}^a \sum_{j=1}^n y_{ij}^2 - \frac{y_{..}^2}{N} \end{aligned} \quad (6.13)$$

and

$$SS_{Treatments} = \sum_{i=1}^a \frac{y_{i.}^2}{n} - \frac{y_{..}^2}{N} \quad (6.14)$$

where $y_{..}$ is the grand total of all the observations, $\bar{y}_{..}$ represents the grand average of all observations, $y_{i.}^2$ represents the total of the observations under i th treatment, $N = an$ is the total number of observations. Degree of freedom (DF) of $SS_T = N - 1$, DF of $SS_{Treatment} = a - 1$, and DF of $SS_E = a(n-1) = N - a$. The error sum of squares is

$$SS_E = SS_T - SS_{Treatments}$$

The statistical analysis to test whether the null hypothesis with no difference in treatment means uses the ratio of:

$$F_o = \frac{MS_{Treatments}}{MS_E} = \frac{SS_{Treatments} / a - 1}{SS_E / (N - a)} \quad (6.15)$$

H_0 is rejected if

$$F_o > F_{\alpha, a-1, N-a}$$

The calculation of analysis of variance is usually carried out as in the following table:

Table 6.12 Analysis of variance for single-factor, fixed effects model

Source of variation	Sum of squares	Degree of freedom	Mean square	F _o
Between treatments	$SS_{Treatments}$	$a - 1$	$MS_{Treatments}$	$F_o = \frac{MS_{Treatments}}{MS_E}$
Error (within treatments)	SS_E	$N - a$	MS_E	
Total	SS_T	$N - 1$		

The adequacy of the model is then checked by carrying out a normal probability plot diagram of residuals or plotting residuals vs. fitted values. The error has to be normal and independently distributed while the variance has to be constant.

The ANOVA test above examined the equality of mean treatments but could not exactly state the difference between treatments. A multi comparison of treatments can be obtained by performing other tests such as Scheffe’s method, or Duncan’s multiple range test. Montgomery (1991) described many methods applied for multiple comparisons.

6.2.3 Factorial design

To study the effects of two or more factors, factorial design is the most efficient procedure since it investigates all possible combinations of the levels of the factors. Factorial design furthermore, can detect any interaction between factors. There are many types of factorial design. The study only involved two-factor factorial design and 2^k factorial design.

i. The two-factor factorial design

The two-factor factorial design involves two factors of A at a levels and B at b levels. The statistical model is:

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk}$$

$i = 1, 2, \dots, a$ $j = 1, 2, \dots, b$ $k = 1, 2, \dots, n$

(6.16)

where $(\tau\beta)_{ij}$ is the effects of the interaction between τ_i (factor A) and β_j (factor B). The analysis of variance includes calculation of the effect of treatment A, treatment B and interaction between A and B. Table 6.13 shows the procedure of calculation of two-factor factorial design.

Sums of squares (SS) are calculated as:

$$SS_T = \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n y_{ijk}^2 - \frac{y_{...}^2}{abn} \tag{6.17}$$

$$SS_A = \sum_{i=1}^a \frac{y_{i..}^2}{bn} - \frac{y_{...}^2}{abn} \tag{6.18}$$

$$SS_B = \sum_{j=1}^b \frac{y_{.j.}^2}{an} - \frac{y_{...}^2}{abn} \tag{6.19}$$

$$SS_{Subtotals} = \sum_{i=1}^a \sum_{j=1}^b \frac{y_{ij.}^2}{n} - \frac{y_{...}^2}{abn} \tag{6.20}$$

and $SS_{AB} = SS_{Subtotals} - SS_A - SS_B \tag{6.21}$

$$SS_E = SS_T - SS_{Subtotals} \tag{6.22}$$

Table 6.13 The analysis of variance for two factor factorial design, fixed effect model

Source of variation	Sum of squares	Degree of freedom	Mean square	F _o
Treatment A	SS _A	<i>a</i> - 1	$MS_A = \frac{SS_A}{a - 1}$	$F_o = \frac{MS_A}{MS_E}$
Treatment B	SS _B	<i>b</i> - 1	$MS_B = \frac{SS_B}{b - 1}$	$F_o = \frac{MS_B}{MS_E}$
Interaction AB	SS _{AB}	(<i>a</i> -1)(<i>b</i> -1)	$MS_{AB} = \frac{SS_{AB}}{(a - 1)(b - 1)}$	$F_o = \frac{MS_B}{MS_E}$
Error	SS _E	<i>ab</i> (<i>n</i> -1)	$MS_A = \frac{SS_E}{ab(n - 1)}$	
Total	SS _T	<i>N</i> - 1		

ii. The 2^k factorial design

The most important factorial design is a 2^k factorial design which involves *k* factors each at only two levels. This design is powerful in the early stages of experimental

procedure where many factors have to be investigated. It also provides the smallest number of runs to study the effects of factors.

In this study, the 2^k factorial design was used to investigate the application of reverse osmosis (2^2 factorial design) and its pre-treatment (2^3 factorial design). To shorten the explanation, this part will only describe the 2^3 factorial design *i.e.* factorial design with three factors (A, B, and C) at two levels as ‘low’ or (–) and ‘high’ or (+). There would be eight treatment combinations in the experiment in which its notation and factorial effects are illustrated in Table 6.12.

The analysis of variance procedure is similar to those with two-factor factorial design by testing the value of F_0 ratio of $MS_{factors}/MS_{Error}$. The interaction is tested however, hierarchicals from the highest order interaction, *i.e.* the interaction of ABC, then BC, AC and AB. If the highest order interaction is not significant, the analysis is repeated after omitting the least non-significant highest order interaction term until all the terms in the model are significant and the model remains hierarchical.

Table 6.14 Notation & algebraic signs for calculating effects in 2^3 design

Run	A	B	C	Treatment combinations	Factorial Effects							
					I	A	B	AB	C	AC	BC	ABC
1	-	-	-	(1)	+	-	-	+	-	+	+	-
2	+	-	-	a	+	+	-	-	-	-	+	+
3	-	+	-	b	+	-	+	-	-	+	-	+
4	+	+	-	ab	+	+	+	+	-	-	-	-
5	-	-	+	c	+	-	-	+	+	-	-	+
6	+	-	+	ac	+	+	-	-	+	+	-	-
7	-	+	+	bc	+	-	+	-	+	-	+	-
8	+	+	+	abc	+	+	+	+	+	+	+	+

6.3 Analytical Methods

6.3.1 Soil analyses

i. Sample preparation & storage

Soil samples were dried at room temperature and sieved to pass through a 2mm sieve mesh. Stones and other large materials such as rubbish, roots/plants were rejected. The soil was also crushed on the sieve until only stones larger than 2mm were retained. The soil sample was collected after riffing through the 15mm divider to produce the required amount.

Soil samples were stored in double polythene plastic bags in which some water drops were maintained to keep soil moisture and minimise evaporation (Williams and Gray, 1973). Soil samples from the column were directly used without any sieving but after air drying at room temperature.

ii. Analytical methods

Soil analyses were carried out unless stated according to procedures described in Standard Methods (1985). Analyses and methods used were:

- a. Soil moisture was determined using gravimetric method by measuring the difference between weight after 3 hours drying at 105°C (Allen, 1989)
- b. Loss on ignition as an estimation of organic content was analysed by further drying of soil sample from the moisture analysis in a 550°C furnace for 2 hours (Allen, 1989)
- c. pH of the soil was determined using pH meter after mixing with 0.001M CaCl₂ (Ecker & Sims, 1997)
- d. Heavy metals (excluding As) were measured by Unicam Atomic Absorption Spectrometer (AAS) after concentrated HNO₃ extraction
- e. Arsenic was analysed by Induced Couple Plasma (ICP) after concentrated HNO₃ extraction

- f. Nitrogen content was analysed by Kjeldahl procedure.
- g. Sulphate (SO_4^{2-}) measurement was carried out using Ion Chromatograph - DIONEX after mixing/extraction with NH_4 Acidified Acetate and activated carbon and filtration using a Whatmann no. 42 filter (Singh et al., 1997)

The operation condition of Ion Chromatograph DIONEX are as follows:

Sample size: 20 μl
 Carrier gas: Nitrogen
 Carrier gas pressure: 90 psi / 9 psi
 Detector: Thermal Conductivity (TC)
 Detector range: 30 μS
 Background conductivity: 13-14 μS
 Eluent: Combined Na_2CO_3 and NaHCO_3 solution
 Pump flow rate: 2.0 ml/min
 Column packed with latex resin beads.

6.3.2 Leachate and liquid analyses

The analytical methods applied to leachates from the site and the column tests were normally carried out according to procedures outlined in Standard Methods (1985).

The method and instruments used were:

- a. pH measured by digital pH meter Jenway 3310
- b. Heavy metals measurement excluding As were carried out by Unicam Atomic Absorption Spectrometer
- c. Arsenic measurement was performed by Induced Couple Plasma
- d. Sulphate (SO_4^{2-}) was determined by Ion Chromatograph DIONEX with operation condition as above
- e. Sulphide was analysed by the Methylene Blue method
- f. COD measurement was carried out in a Hach Dichromate reflux apparatus
- g. Volatile fatty acids were determined by Unicam 610 series with autojector and PU 4811 integrator. The operation conditions were:

Sample size: 1 μl solution of acids in 2.5 N phosphoric acid
 Carrier gas: Nitrogen
 Detector: Flame Ionisation Detector (FID)
 Detector temperature: 180°C
 Injection type: Syringe-On column
 Injection temperature: 180°C
 Column temperature: 140°C
 Pump flow rate: 2.0 ml/min

Column: 2m x 2mm I.D. glass packed with 10% AT-1000 on 80/100 Chromosorb W-AW

- h. Turbidity was measured by Hach turbidimeter Model 2100A,
- i. Colour was analysed by Lovibond Nessleriser MK1, and
- j. Conductivity measurement was by conductivity meter.

6.3.3 Gas analyses

CO₂ and CH₄ were measured by Gas Chromatograph Becker model 403 with Unicam 4815 computing integrator. The conditions were as follows:

Sample size: 1ml gas mixture
Carrier gas: Helium
Detector: Thermal Conductivity (TC)
Injection type: Syringe-On column
Injection temperature: 60°C
Column temperature: 55°C
Pump flow rate: 50 ml/min
Column: 2m x 4mm I.D. metal packed with Porapak Q.

Hydrogen sulphide gas from the soil column test was measured by Gas Chromatograph Pye Unicam Series 104 with Hewlett Packard 3395 integrator. The operating conditions were:

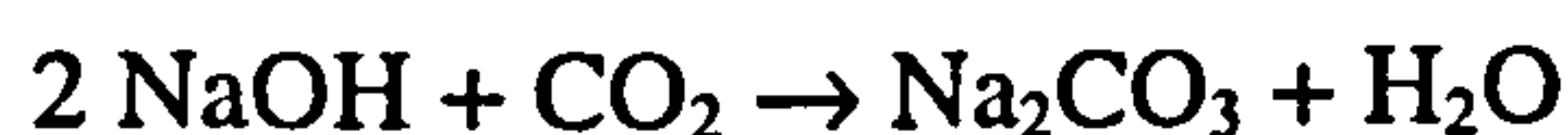
Sample size: 1ml gas mixture
Carrier gas: Helium
Detector: Thermal Conductivity (TC)
Injection type: Syringe-On column
Injection temperature: 110°C
Column temperature: 110°C
Pump flow rate: 50 ml/min
Column: 1.5m x 4mm I.D. glass packed with 100-120 mesh Porapak T.

6.3.4 Microbial analyses

The analyses included measurement of microbial activity using CO₂ evolution, microbial growth, and microbial populations by direct counts and plate counts. Gram staining was also used to investigate the bacterial types and morphology.

i. Respiration measurement

Microbial respiration in soil slurries and the columns were by measuring the released CO₂ gas which was trapped in the air stream by alkali (1N NaOH).



Before titration BaCl₂ 5% was added to precipitate the CO₃⁼ as BaCO₃ and excess NaOH was backtitrated with acid (1N H₂SO₄). The weight of CO₂ absorbed was equal to the volume of NaOH converted to Na₂CO₃ and multiplied by 0.022 (Dennis, 1971).

ii. Microbial growth

As mentioned in a previous section (§ 6.1.2), the microbial growth was estimated by measuring the turbidity of the soil extract after addition of the material under investigation. The agitation of mixtures was carried out in a Unitron CH-4103 Bottmingen - digital orbital shaker. Turbidity was assessed by measuring absorbance in a Bausch & Lomb Spectronic 501 spectrophotometer at 530nm.

iii. Direct counting

The microbial populations in soil samples were estimated by direct count microscopy on soil extract samples. The soil extract was obtained by agitating the soil sample with distilled water using Tween 80 as the surfactant. The acridine orange direct count procedure, described by Hobbie et al. (1977), was used. The counting of stained bacteria was carried out in an epifluorescent microscope (Zeiss 47 60 05 - 9901) using a filter (Zeiss 46 63 01 - 9901 coded FT 510, LP 520) to give blue excitation at 450-490nm at 1000x magnification.

Soil extracts for microbial counting were obtained by shaking the soil mixture in a Voss shaker at 1500rpm for 20 min.

iv. Plate counting

Pour plates for viable heterotrophic bacterial counting was carried out using R2A agar at 20-22°C. Heterotrophic plate count (HPC) enumeration was carried out after 7 days. To count the colonies, a Gallen Kamp colony counter was used. The pour plate procedure was outlined in Standard Methods (1985). Streak plates on R2A agar was also attempted in order to grow some colonies for gram staining.

v. Gram staining

Gram staining was used for some of the bacterial colonies from the streak plate count and mixtures from the microbial growth tests. The gram staining procedure is described in Standard method (1985). An Olympus BH2 microscope was used to view the staining.

CHAPTER 7

RESULTS AND DISCUSSION OF BIOREMEDIATION OF SLURRIED SOIL

7.1 Background

The objective of the bioremediation study was to investigate the treatability of the contaminated soil under investigation using either an aerobic or anaerobic biological treatment process with the addition of nutrients and/or glucose as a carbon source. The aims were to assess the documented loss of contaminants which were accompanied by the growth of the microbial community in the soil. The bioremediation study was carried out using three types of experiments, namely soil slurry, microbial growth and soil column tests. These will be included subsequently in three chapters.

This chapter will cover results and discussion in experiment with soil slurry. The objective of slurry test was to investigate the environment suitable for reduction of contaminants studied. The term of environment involved conditions (aerobic or anaerobic) and appropriate nutrients added. There were three types of method investigated namely aerobic (Method I), anaerobic (Method II) and respirometer tests (Method III). Through the experiment the nutrients added varied. Aerobic method only covered N and P sources at different concentration. The anaerobic test also included N only and N, P plus trace elements besides N and P sources. The third method with respirometer also put into consideration of P only source. Samples 1, 2 and 3 were the soils studied in Methods I and II and Soils 4 and 5 were used in Method III. Therefore, discussion also covered comparison of methods for the benefit of future soil studies. The experiments involved responses to pH, sulphate, zinc and manganese concentration and some other reactants from the process.

A pull-out explanatory note on the methods and conditions or treatments investigated is available after Chapter 9 (page 176) as a summary of the terminology used in the results and in discussions of the experiments.

7.2 Keys of Reactions Involved

7.2.1 Microbiological reactions

In this study, sulphur proceeds two possible microbial processes, namely mineralization or immobilization or reduction. The mineralization process of sulphur produces sulphate from the soil and therefore raises sulphate concentrations in soils. Sulphur immobilization results in less sulphate in the leachate or the soil studied.

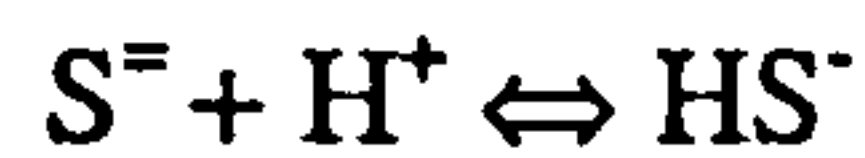
To decrease the sulphate concentration as the aim of this study, two mechanisms may be considered. The first is dissimilatory sulphate reduction where sulphide and alkalinity were produced. Dissimilatory sulphate reduction occurs under anaerobic condition in which sulphate acts as electron acceptor by sulphate reducing bacteria that can couple the oxidation of reduced organic or inorganic compounds to the reduction of sulphate for bioenergetic purposes (Colleran, et al., 1995). Dissimilatory mechanism could also end with precipitation of some metals as metal sulphide. The optimum pH for sulphate reduction is around pH 7 (neutral). The second mechanism is assimilatory sulphate reduction which results in a higher sulphur content in the biomass. Sulphur is taken up by bacterial species prior to incorporation into biological compounds such as cysteine, methionine, co-enzyme A, etc. (Colleran, et al., 1995). This can be carried out in aerobic or anaerobic condition (Killham, 1994).

In terms of sulphate transformation, assimilatory sulphate reduction requires 1 mg of sulphate to supply the growth of approximately 200 mg *Klebsiella aerogenes* cells whereas in dissimilatory sulphate reduction 1 mg of sulphate yields 0.5 to 1.0 mg of cell mass for dissimilatory *Desulfovibrio* sp. (Colleran, et al., 1995).

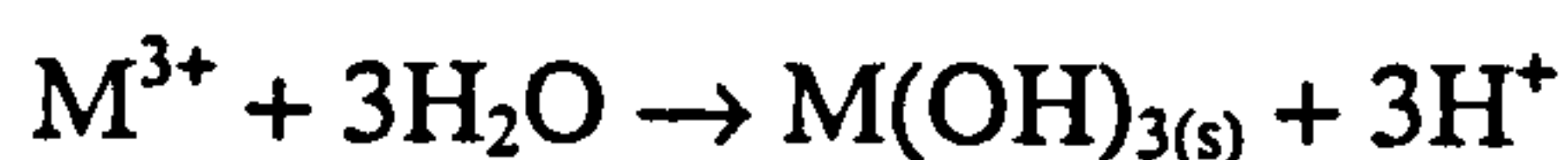
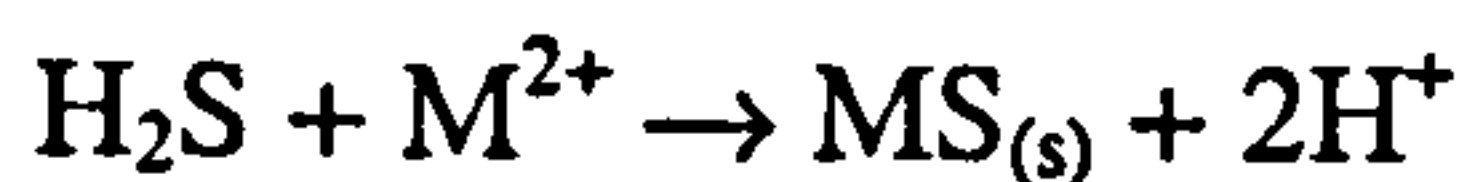
The activity of microorganisms in those mechanisms was estimated by measuring the CO₂ production from soil slurry in the reactor.

7.2.2 Chemical reactions

The reaction of sulphate reduction process by anaerobic sulphate reducing bacteria is as follows:



The relationship H_2S , HS^- and S^{2-} is pH dependent. At pH value of 8 and above most reduced sulphur presents in solution as HS^- and S^{2-} ions and the amount of free H_2S is so small. At pH level below 8 the equilibrium shifts to form unionised H_2S and is about 80% completed at pH 7. Under such condition the partial pressure of hydrogen sulphide generates odour problem (Sawyer et al., 1994). In the other hand, the H_2S can react to precipitate metals as insoluble metal sulphides (Smith et al., 1994). The alkalinity production increases the pH which results in metal removal through the formation of insoluble metal hydroxides or oxides. The reactions involved are:



7.3 Aerobic Slurry Tests (Method I)

In this method 4 flasks were used, namely:

- i. Flask 1: as a control, soil slurry aerated without nutrient addition;
- ii. Flask 2: soil slurry aerated and nutrient added;
- iii. Flask 3: soil slurry with the addition of activated sludge, aerated and nutrient added;
- and
- iv. Flask 4: soil slurry and activated sludge, with nutrients, without aeration.

The activated sludge used for the experiment was obtained from a domestic wastewater treatment plant.

Aerobic method results included the response of pH, sulphate, zinc, CO_2 , and volatile suspended solids as a percentage of suspended solids to estimate the microbial proportion of the system.

7.3.1 Results of aerobic soil slurry tests

i. pH changes during experiments

The pH in the control Flask (No. 1) was always greater than that in the other test flasks. The pH in the control remained relatively constant throughout the period of the experiment as shown in Figure 7.1. On the other hand, the pHs in Flask 4 (without aeration) were in most cases the lowest. Between this, the pHs in Flasks 2 and 3 (which were aerated) and had nutrient added were higher than in Flask 4 but were always lower than in the control flask. This suggested that different aeration levels and environments in the soil slurry gave significantly different pH responses.

The experiment included an investigation into the changes in pH if the nutrient addition was increased. The pH of the system was shown to decrease as the nutrient level increased. The reduction in pH was not similar for all soil samples studied. Statistically, in Soil 1, the Nutrient 1 (N1) increase firstly affected the pH in Flask 2 for the higher level of nutrient added, the pH in Flask 3 was also reduced, later becoming, at 5% significance, no different from Flask 2. In Soil 2, the pH reduction however, was not similar in a way that the pH of Flask 2 and 3 were only lower than control after '10ml' nutrient addition (the highest amount). In Soil 3, all treatment flasks were showing a pH lower than the control after '2.5ml' nutrient addition. Furthermore, the reduction of pH in Flasks 2 and 3 was much greater than that in Flask 4.

This indicated that the amount of nutrient added affected the pH response and in turn depended on the soil samples. However, it was always noted that the control flask had the highest pH, followed by the aerated+nutrient added flask, while the lowest pH occurred in Flask 4 (without aeration). No aeration in Flask 4 was likely to result in anaerobic conditions. This could encourage the sulphate reduction process to occur and produce H^+ which would decrease the pH.

ii. Results of CO₂ evolution study

CO₂ evolution from the soil slurry in an aerobic environment (Method I) was measured by titration of CO₂ in the NaOH trap using H₂SO₄. Figure 7.2 shows the CO₂ evolution in each soil sample. The graphs demonstrate that CO₂ evolution in the flask without aeration (Flask 4) was significantly lower than those with aeration. The amount of CO₂ evolved from Flask 4 in all soil samples could be considered as negligible compared to Flasks 1-3. Comparisons of Flasks 1-3 results on the effect of nutrient added, showed that only Soil 1 in Flask 3 (with activated sludge) exhibited a higher CO₂ production than in control (Flask 1) with the 'Once-supplied', '2.5ml' and '5ml' nutrient addition. For Soils 2 and 3, CO₂ evolution was no higher in those flasks with nutrient addition.

This outcome was also supported by an analysis of variance and multiple comparisons in that Flask 4 always produced the least amount of CO₂. It was thus assumed that in aerobic conditions, nutrient addition did not significantly increase respiration. The CO₂ measured was likely to have been contributed from the aeration process. It may also be relevant to suggest that the method was not sensitive to small changes in CO₂ evolution when comparing the aerated flasks.

iii. Results of changes in sulphate concentration

The study was intended to investigate the reduction in sulphate concentration in the soils and the leachate from the contaminated site. Dissimilatory sulphate reduction by sulphate reducing bacteria occurs in anaerobic conditions whereas assimilatory sulphate reduction may also be possible in aerobic environments. The slurry test therefore, also included an investigation into sulphate changes in an aerobic environment.

As shown in Figure 7.3 for Soil 1 the sulphate concentrations in the nutrient addition flasks was not lower than those in control. The analysis was by paired t-tests of segmented data (according to the amount of nutrient added) and the sulphate in those treatments with nutrient addition were found to be significantly lower in those flasks with activated sludge (Flasks 3 and 4) at '5ml' nutrient, and Flasks 2 and 3 at '10ml'

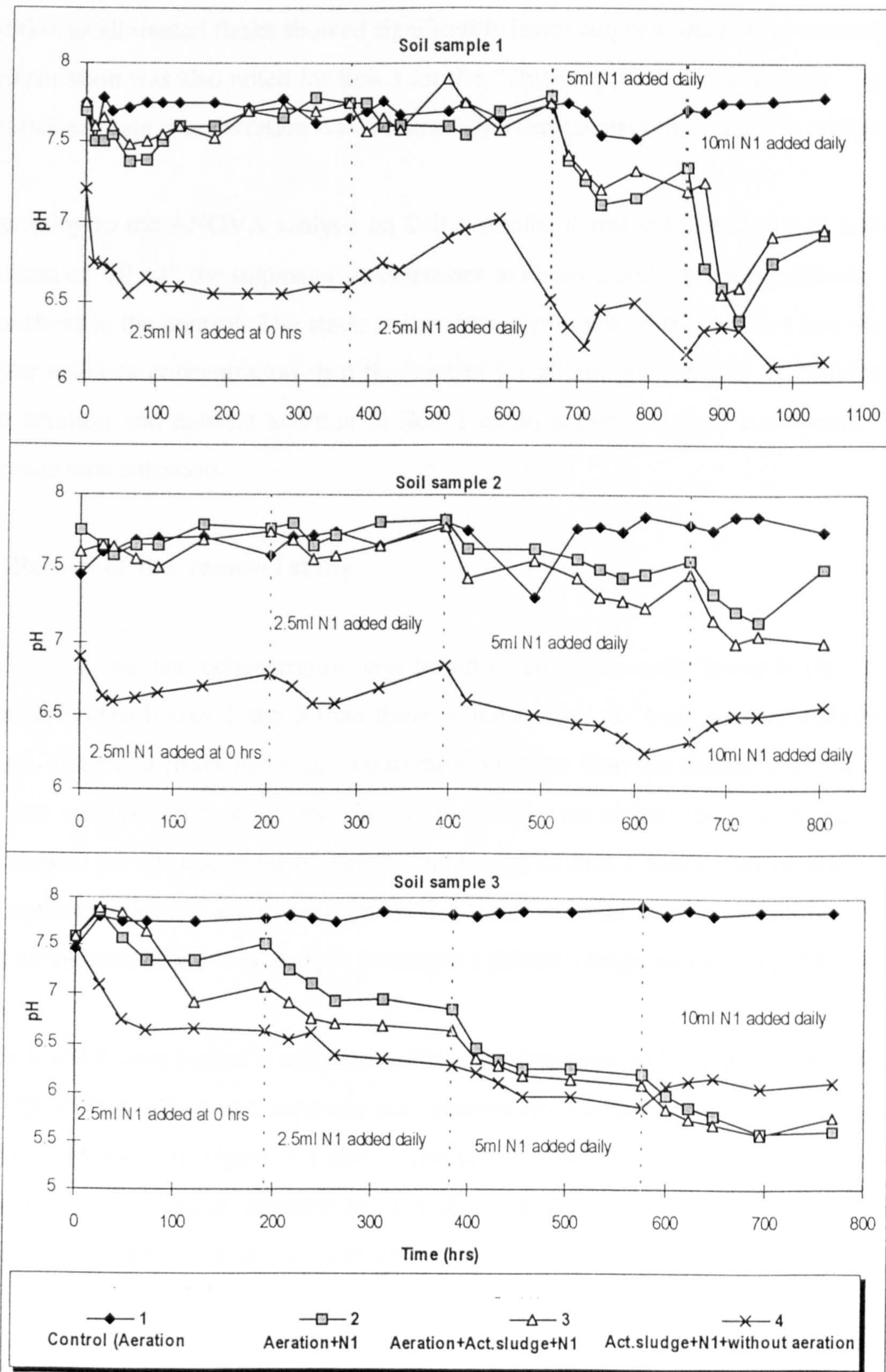


Figure 7.1 pH response in aerobic soil slurry tests (Method I)

nutrient addition. For Soil 2, sulphate was found to be lower in some of the flasks at all nutrient levels. However, an interesting result was demonstrated in the '5ml' nutrient addition as all treated flasks showed significantly lower sulphate than in the control flask. This situation was also noted for Soil 3 for the 'Once-supplied' nutrient. This suggests that the sulphate concentration was reduced by a certain amount of nutrient addition.

According to the ANOVA analysis on Soil 1 results, it was calculated that at a nutrient addition of '10 ml', the sulphate concentrations in Flasks 2 and 3 were significantly lower than those in the control. The statistical analysis also revealed that Flask 4 had always a higher sulphate concentration than the control for all nutrients levels. It could be said that aeration and nutrient addition to Soil 1 could possibly lead to a reduction in the sulphate concentration.

iv. Results of zinc removal study

For Soil 1, the zinc concentration was found to be significantly lower in the nutrient-aerated-treated Flasks 2 and 3 than those in the control at '5ml' nutrient addition. For Soil 2, all treated flasks had a zinc concentration lower than the control after the '2.5ml' nutrient addition. In contrast, the zinc concentration was always found to be higher than the control for all treatments of Soil 3. The results of Soil 1 and 2 may be attributed to the optimum nutrient level which affected the zinc concentration whereas Soil 3 which was an uncontaminated soil sample presented a different response (see Fig. 7.4).

Soils 1 and 2 were higher in sulphate and zinc content than Soil 3. Figure 7.4 also shows that Zn in Flask 4 (without aeration) was dramatically increased after '2.5 ml' and '5 ml' nutrient addition. In Figure 7.1 shows the pH of Flask 4 fluctuated at this period of experiment. This could possibly explain that nutrient addition changed the pH and consequently altered the solubility of Zn.

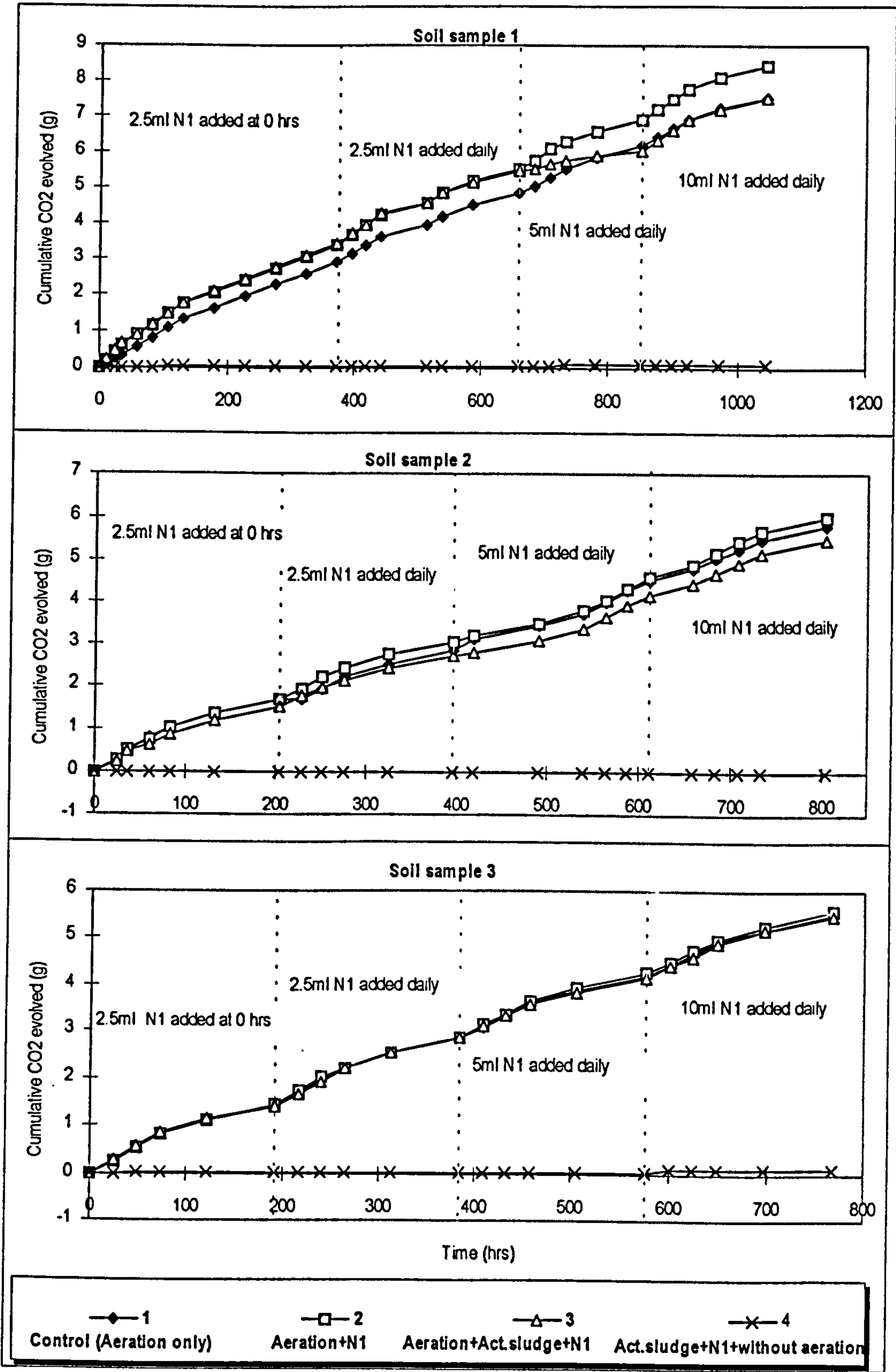


Figure 7.2 Cumulative CO₂ evolved -aerobic slurry tests (Method I)

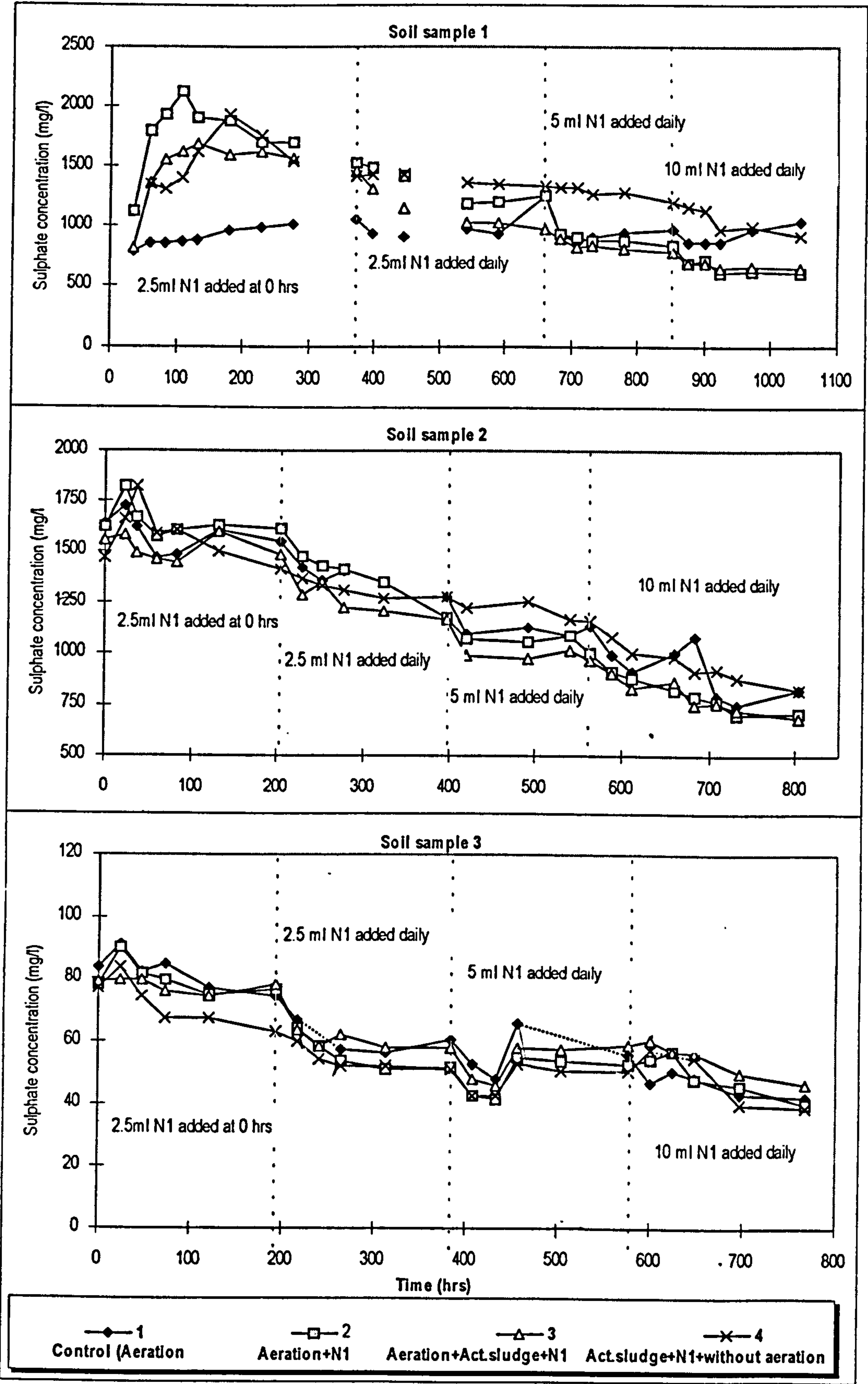


Figure 7.3 Sulphate concentration - aerobic soil slurry tests (Method I)

v. Results of volatile suspended solids content

Although the VSS determination is not an exact reflection of the microbial population, the method is widely used to estimate the microbial consortium in a biological wastewater treatment plant. The hypothesis is that the VSS will be increased if the microbial population is able to adapt and grow in the studied environment.

The statistical analysis by paired t-test of Soil 1 VSS results showed that the VSS levels in the treated flasks were always greater than those in the control at all nutrient level additions. However, according to the ANOVA analysis only the '5ml' nutrient addition exhibited the treatment differences where all treated flasks were greater in VSS content than in the control. The highest VSS content for Soil 1 was noted in Flask 4 (without aeration) at all nutrient levels except for 10ml addition where the highest was found to be in Flask 3.

The paired t-test of VSS results of Soil 2 also demonstrated similar conclusions to Soil 1. All, except in Flask 2 at '5ml' nutrient, VSS levels in the treated slurry were significantly higher than those in the control for all nutrient levels. The highest VSS content was found in Flask 3. On the contrary, this was not the case for Soil 3 where none of the test flasks and nutrient levels resulted in a VSS content greater than those of in the control flask. The reason could be that Soil 3 did not contain sufficient sulphate for growth. Sulphur is usually required around 1% of cell mass (Metcalf, 1993).

Although the VSS content was higher in Flask 4 this was not accompanied by higher CO₂ evolution. The reaction without aeration seemed to be the appropriate condition for the indigenous microbial population to grow. However their activity in term of CO₂ measured was very low. This could be caused by the failure of CO₂ measurement that only detected the CO₂ source from the aeration.

However, it was interesting to note findings by Chander & Brookes (1991) and Fließbach et al. (1994). They found that in the environment with high heavy metals concentration, the biomass content was low and the CO₂ evolved was high. They explained that this was related with division of the energy between growth and cell

maintenance. Under stress condition such as with high heavy metals content, extreme pH and temperature and salinity, the division became uneasy. The energy was more for cell maintenance than cell synthesis.

7.3.2 Summary of aerobic soil slurry test results

Aerobic slurry tests showed that the pH response in the flask could detect differences in treatment and nutrient level conditions. The pH in the control flask was always found to be higher than in the treated sample flasks. On the other hand, flasks without aeration exhibited the lowest pH in all soil samples. It was also observed that nutrient addition affected pH. As the nutrient level increased the pH decreased. However, the rate of fall of pH after nutrient addition was different for each soil sample.

With regard to CO₂ evolution, the method was not sufficiently sensitive to detect differences between treatments. It could only show that the non-aerated sample always exhibited the lowest CO₂ evolution and could be considered as negligible. This also suggested that the method required improvement for the measurement of CO₂ evolution.

The sulphate concentrations in the treated samples were not always lower than those of in the control. However, in some cases, nutrient addition significantly reduced the sulphate level. This confirmed the possible benefit of nutrient addition for sulphate removal. For example in Soil 2, it appeared that '5ml' nutrient addition reduced the sulphate concentration for all treated samples. Similarly for zinc, where Soil 2 with '2.5ml' nutrient addition showed a zinc reduction for all treated samples.

VSS changes in the aerobic test gave good results since it could be seen that for Soils 1 and 2, the VSS content increased as the nutrient was added. In Soil 1, the slurry without aeration resulted in the highest VSS content. In Soil 2, the slurry with activated sludge plus aeration also showed the highest VSS content. In contrast, Soil 3 was not able to produce similar results. This may have been due to the fact that it was not highly contaminated and possessed a different soil structure.

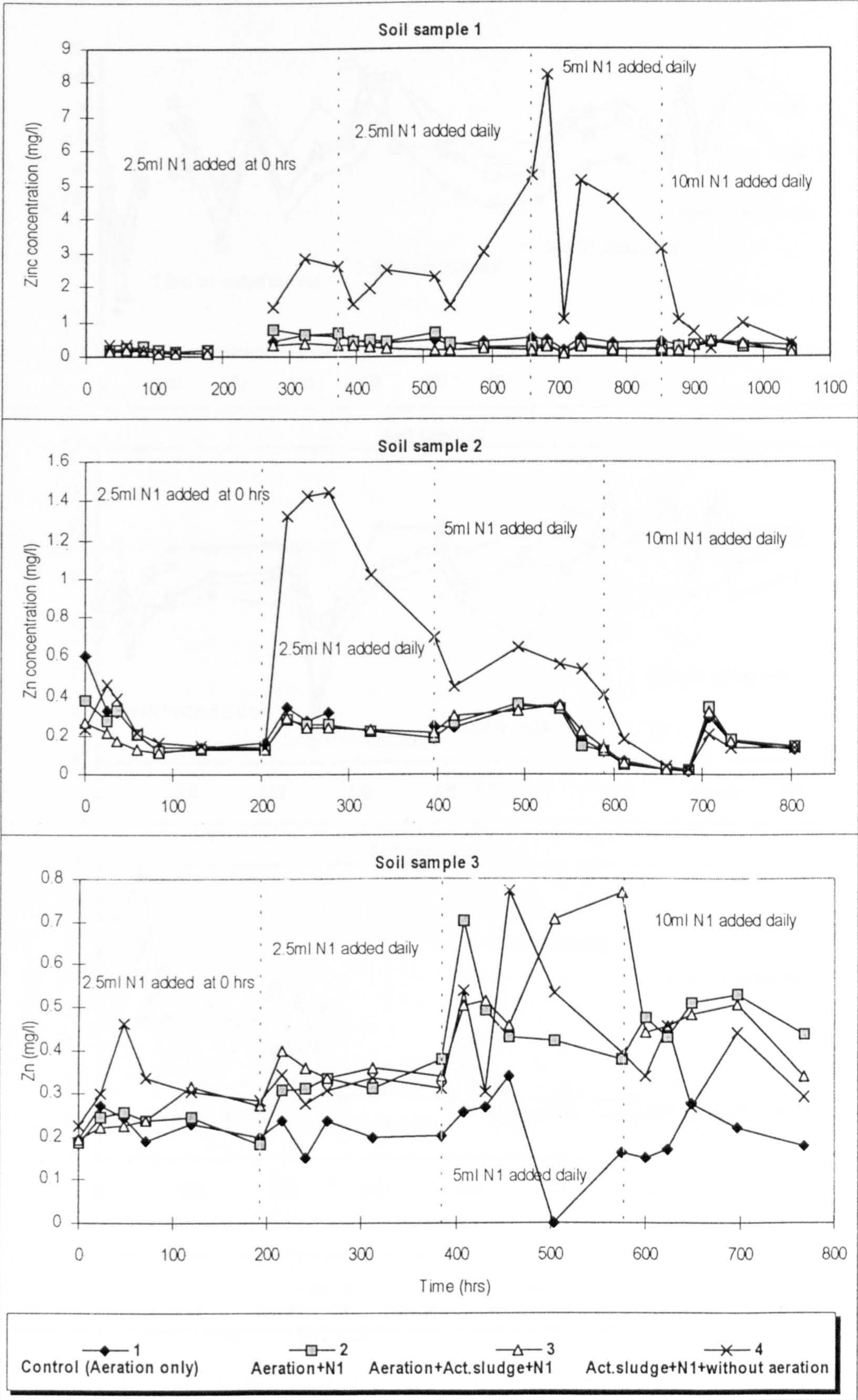


Figure 7.4 Zinc concentration – aerobic slurry tests (Method I)

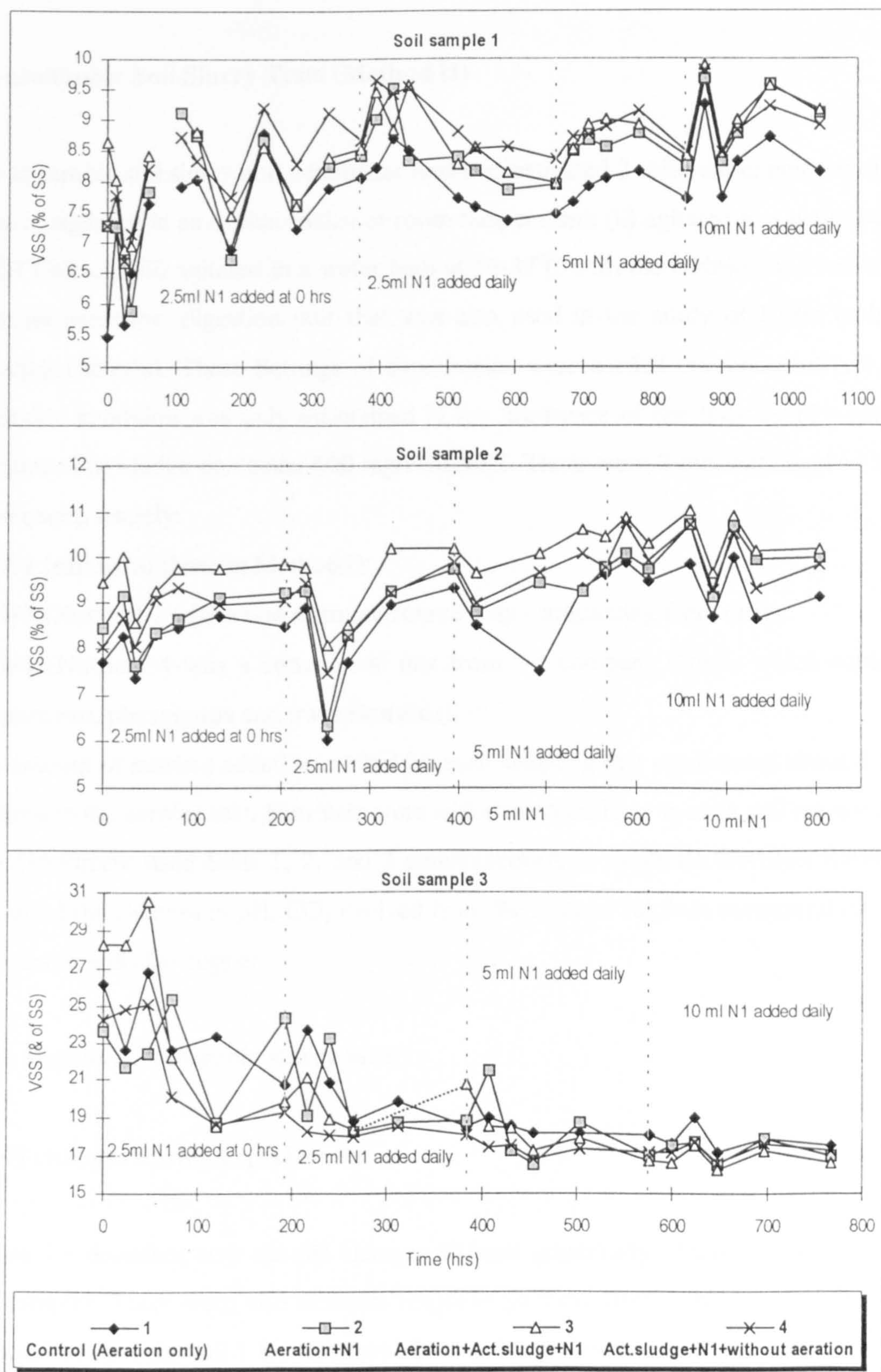


Figure 7.5 Volatile Suspended Solid (VSS) in aerobic slurry test (Method I)

7.4 Anaerobic Soil Slurry Tests (Method II)

The anaerobic soil slurry in the biometer flasks investigated 3 different conditions. These were (i) agitated in an orbital shaker at room temperature; (ii) agitated in a water bath at 36-37°C; (iii) agitated in a water bath at 36-37°C with the addition of 25ml sludge from an anaerobic digestion unit that was also used in the study of SRBs (sulphate reducing bacteria). These Settings of experiments were carried out consecutively. The anaerobic condition was only maintained in the headspace of the flask by nitrogen gas injection. The sludge contained 600 mg/l MLVSS. There were 3 nutrients applied in the experiment, namely:

- N1 (similar to those in Method I);
- N2 (Nutrient 2 which was nitrogen source only - originating from urea);
- N4 (Nutrient 4 was a commercial mix from the company Omex, which contained nitrogen, phosphorus and trace elements).

The amount of nutrient added was 10ml for each sampling that represented about 5 times of those in the aerobic test. Nutrients were added consecutively in each soil reactor flask. The experiment used Soils 1, 2, and 3 simultaneously in duplicate biometer flasks and measured the changes in pH, CO₂ evolved from the system; sulphate concentration, zinc, manganese and also copper.

7.4.1 Results of anaerobic slurry tests

i. pH changes during experiments

Figure 7.6 describes how the pH changes differed graphically according to the various soil samples. There were also different response patterns after daily nutrient addition to each soil sample. In Soil 1 for treatment Setting 1 at room temperature, statistically only the pH after Nutrient 1 (N1) addition showed a significantly lower value than that without nutrient. There was no difference in pH after nutrient addition to Soil 2 whereas only after addition of Nutrient 2 (N2) was there a significant reduction in pH for Soil 3.

In treatment Setting 2 where the flasks were agitated in a water bath at 36°C, the pH changes between soil samples showed different results. All soil samples exhibited a pH higher than those without nutrient addition. In Soil 1, addition of N1 and N2 showed significantly higher pH than those samples without nutrient addition whereas for Soils 2 and 3, N2 and N4 addition resulted in an increased pH. This showed that changes in temperature affected the pH value. The pH was found to be higher in flasks at 36°C after the nutrient addition.

Similarly, in the water bath with the SRB environment (Setting 3), the pHs in those flasks with added nutrient were found to be greater than those without nutrient addition. N2 addition in Soil 1, all nutrients in Soil 2, and N2 and N4 addition in Soil 3, all had a significantly higher pH than those without nutrient addition. In addition, one-way ANOVA analysis of each soil sample showed that there was a significantly different pH response after nutrient addition.

Comparing the effect of treatment settings on pH response, it was found for Soils 1 and 2 that the treatment setting resulted in significant pH differences. The pH of Setting 1 was the lowest, followed by Setting 2, and in the Setting 3 the pH was found to be the highest. For Soil 3, however, the comparisons of pH between Settings revealed that the pH in Settings 2 and 3 were not significantly different but they were higher than the pH in Setting 1.

The slurry pH was found to be high for the anaerobic condition. This presumably was due to temperature increment. White and Gadd (1991) found that sulphide production from the sulphate reducing process was optimum at 20°C. The other factor that could be the cause was the procedure of pH measurement that could not be carried out in strictly anaerobic conditions. The biometer flask should be adapted to more easily measure the pH in anaerobic condition.

ii. Results of CO₂ evolution

Although it was not statistically significant, the CO₂ evolution results for anaerobic tests were able to be compared. N1 addition always showed higher CO₂ evolution than those

without nutrient addition. In Setting 1 (orbital shaker), it was found that N4 addition to Soil 1, and N2 and N4 to Soil 2 resulted in lower CO₂ production than without nutrient. In Soil 3, all nutrient additions demonstrated greater CO₂ production than tests without nutrient addition.

For Settings 2 and 3, similar results were also shown. The CO₂ production after nutrient addition was not statistically different. Graphically however, the N1 addition exhibited a slightly greater CO₂ evolution than other nutrient types for Setting 2. For Setting 3, N4 addition, a slightly higher CO₂ was evolved than for other nutrients. Multiple comparisons by the Dunnet method only detected N2 addition in Soil 3 for Setting 1; N1 in Soil 1 for Setting 2; and N4 in Soil 3 for Setting 3 which produced greater CO₂ than without nutrient addition.

iii. Results of sulphate concentration study

Sulphate, Zn, Mn, and Cu concentrations in the supernatant were measured only once after all sets of experimental runs were completed. This was due to the limited amount of soil slurry available in the flask studied. Figures 7.8 - 7.11 illustrate the results of the anaerobic slurry test (Method II) for these parameters.

Statistical analysis showed that sulphate concentrations in all soil samples were affected significantly by the soil treatment Settings. For Soil 1, anaerobic conditions at room temperature exhibited the lowest sulphate concentration. In Soil 2, addition of SRB sludge and a temperature of 36°C was shown to result in the least sulphate concentration. Soil 3, which was uncontaminated, leached more sulphate under anaerobic conditions at 36°C and with SRB sludge added.

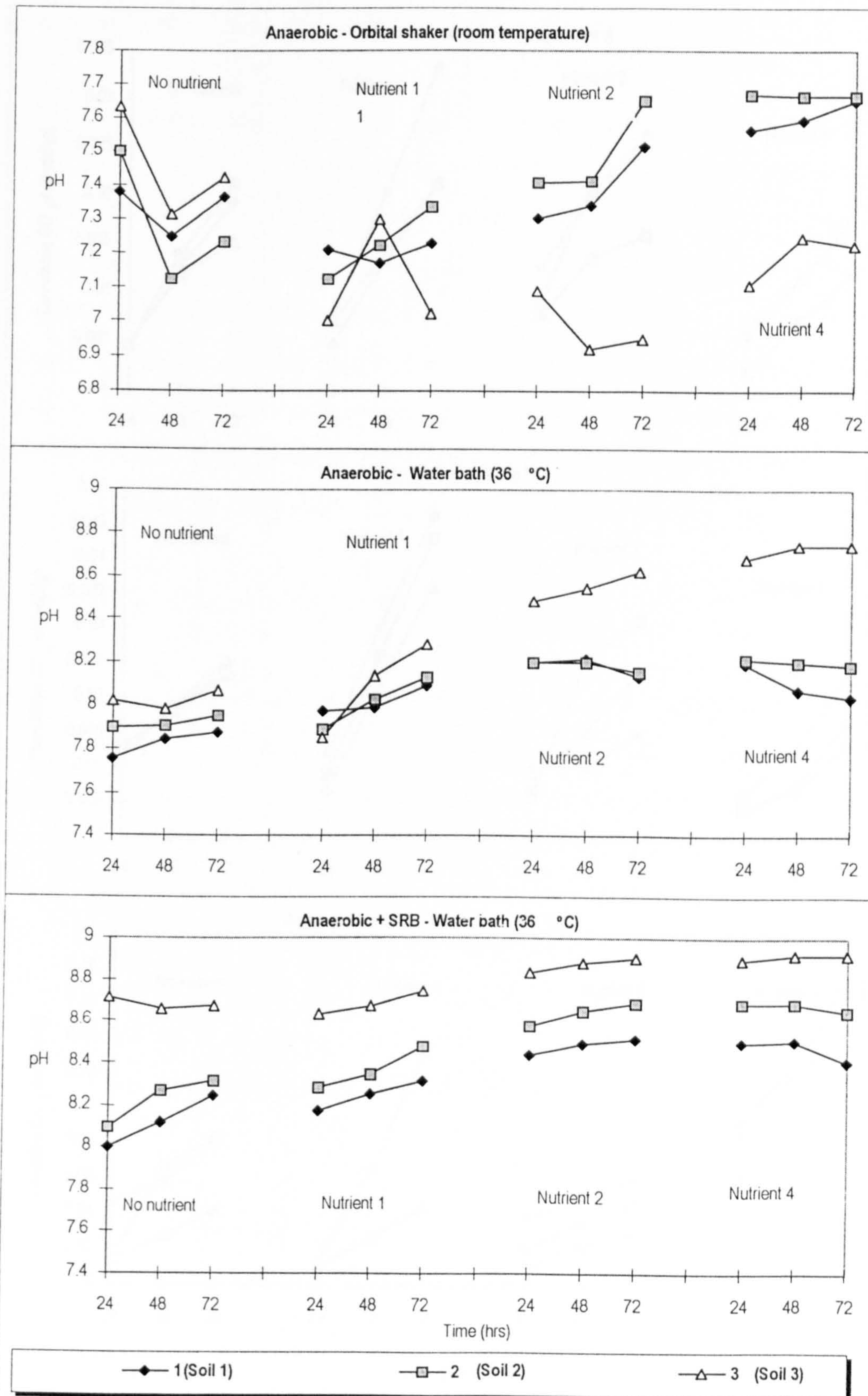


Figure 7.6 pH response - anaerobic slurry test (Method II)

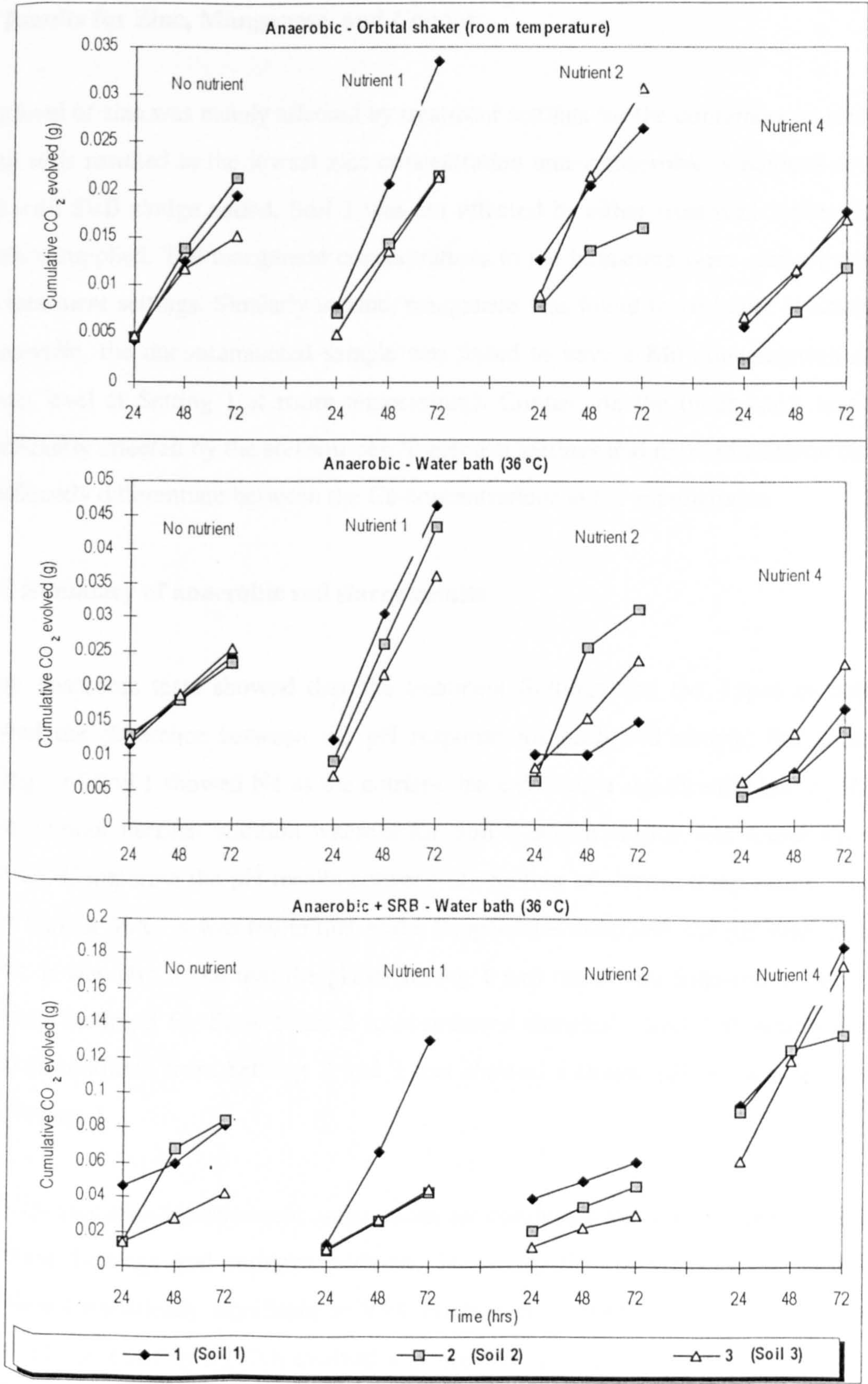


Figure 7.7 Cumulative CO₂ evolution response – anaerobic slurry test (Method II)

iv. Results for Zinc, Manganese, and Copper

The level of zinc was mainly affected by treatment settings for the contaminated samples. Both soils resulted in the lowest zinc concentration under anaerobic conditions at 36°C and with SRB sludge added. Soil 3 was not affected by either treatment setting or the nutrient supplied. The manganese concentrations in the biometers were also affected by the treatment settings. Similarly to zinc, manganese was found to be lower at Setting 3. Meanwhile, the uncontaminated sample was found to have a Mn concentration at the lowest level at Setting 1 (at room temperature). Copper, on the other hand, was only significantly affected by the soil sources. Treatment settings and nutrient addition did not significantly differentiate between the Cu concentrations in the supernatants.

7.4.2 Summary of anaerobic soil slurry results

Using anaerobic tests showed that the treatment Settings and the Types of nutrient showed the difference between the pH response for each soil sample. For example, Setting 1 of Soil 1 showed N1 as the nutrient that exhibited a significantly lower pH than those without nutrient addition whereas for Soil 3 similar results were found after N2 addition. Comparing the pH results between the Setting of 'room temperature' and 'in water bath at 36°C' it was found that as the temperature increased, the pH value became higher. It was also noted that the pH in Setting 1 was the lowest followed by Setting 2 and then Setting 3 for Soils 1 and 2 (contaminated samples). Soil 3 showed a slightly different condition from Settings 2 and 3 that showed a similar pH but was still higher than Setting 1.

The CO₂ evolution measurement under anaerobic conditions showed differences between treatment Settings and nutrient addition. However, the amount detected was not considered statistically significant at 95% confidence interval. In the experiment with SRB addition (Setting 3), CO₂ evolved was greater than the other two conditions. The results for Setting 1, N1 exhibited slightly greater CO₂ production than those without nutrient addition for Soils 1 and 2. It was also shown that N2 and N4 produced a lower CO₂ evolution. For Setting 2, addition of N1 was also found to produce the highest CO₂ evolution whereas in Setting 3, N4 addition resulted in slightly greater CO₂ production.

The sulphate concentration in the contaminated samples was lower in the 'room temperature' system for Soil 1 but adding SRB sludge at a temperature of 36°C the result gave the lowest concentration for Soil 2. Zinc and manganese were at their lower concentration with treatment using SRB sludge and at 36°C for the contaminated samples. For the uncontaminated sample (Soil 3) higher temperature increased Zn, Mn and Cu concentration in supernatants. The reason could be related with the pH that changed the solubility of the metals. On the other hand, Soils 1 and 2 produced lower metal concentration in experiment with higher temperature. Possibly the condition encouraged metals' precipitation as metal sulphide.

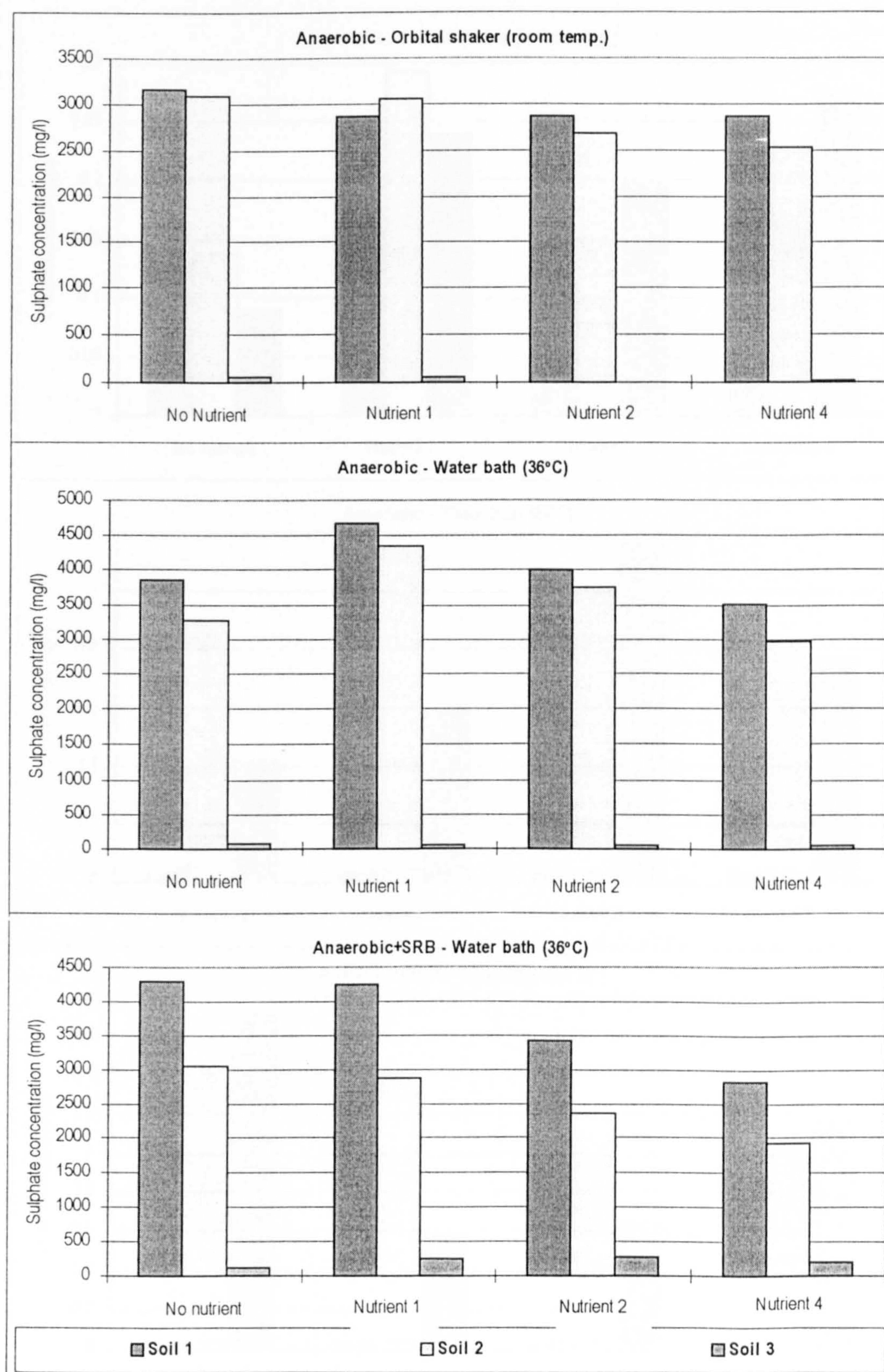


Figure 7.8 Supernatant sulphate concentration – anaerobic slurry test (Method II)

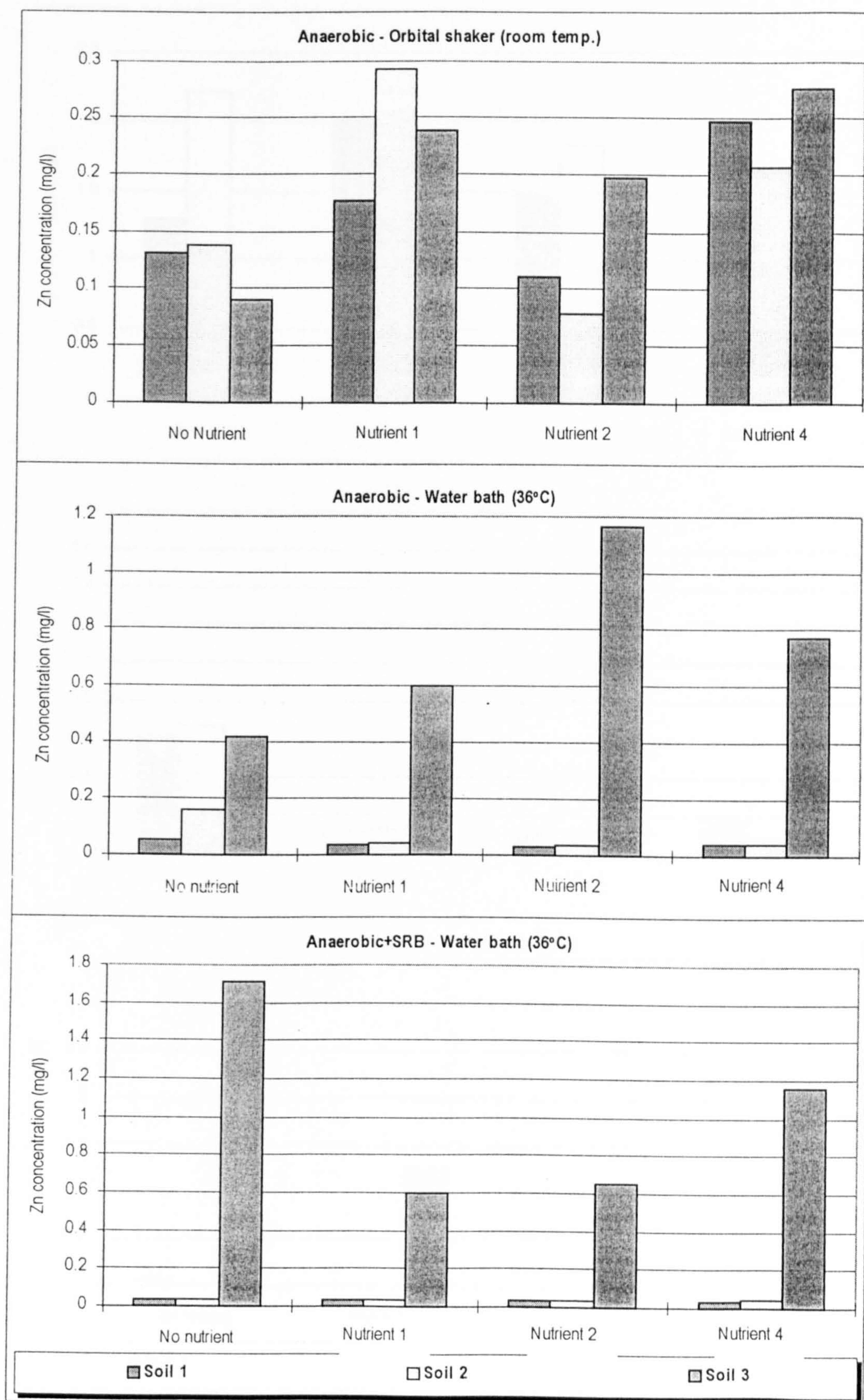


Figure 7.9 Supernatant zinc concentration – anaerobic slurry test (Method II)

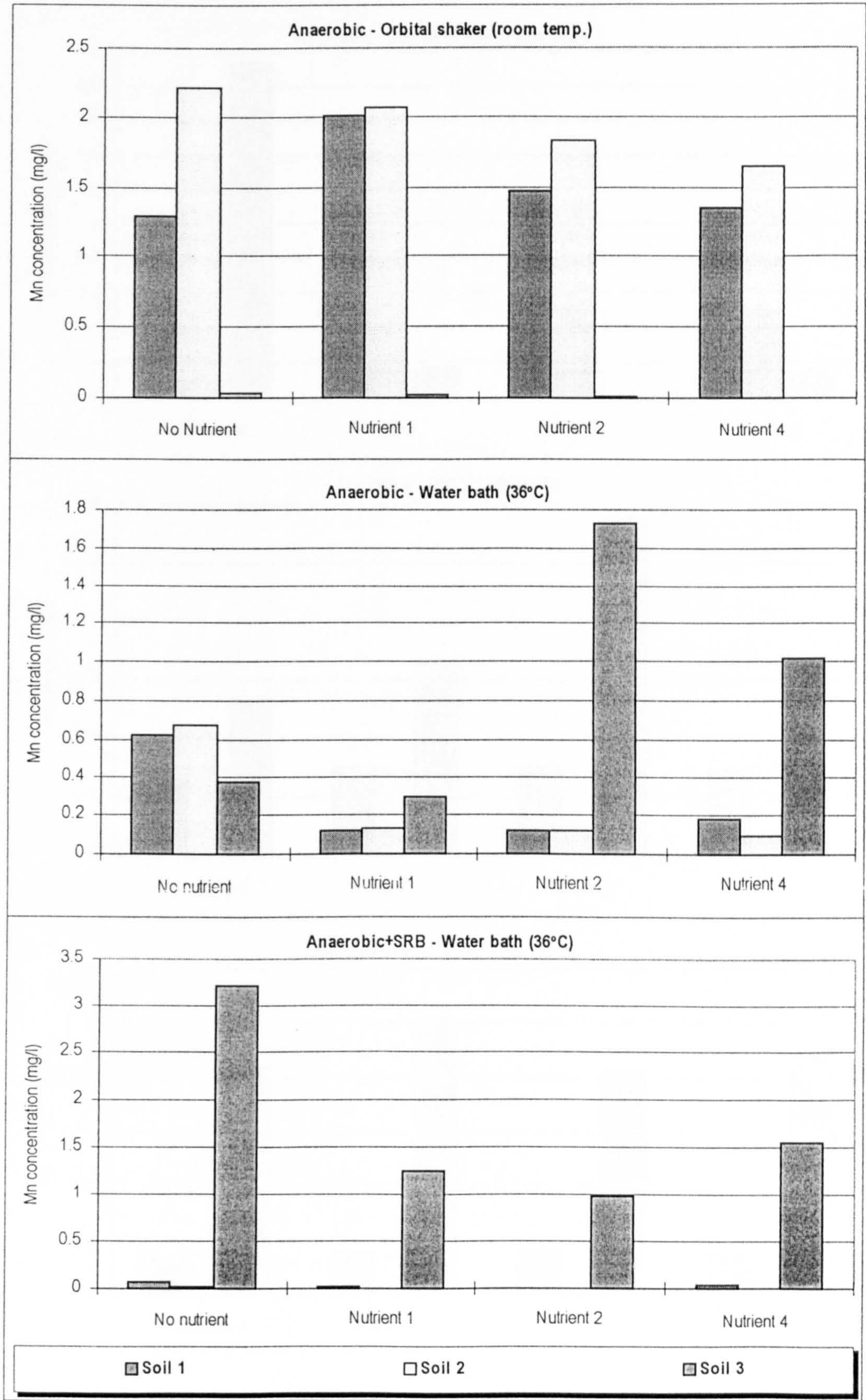


Figure 7.10 Supernatant manganese concentration – anaerobic slurry test (Method II)

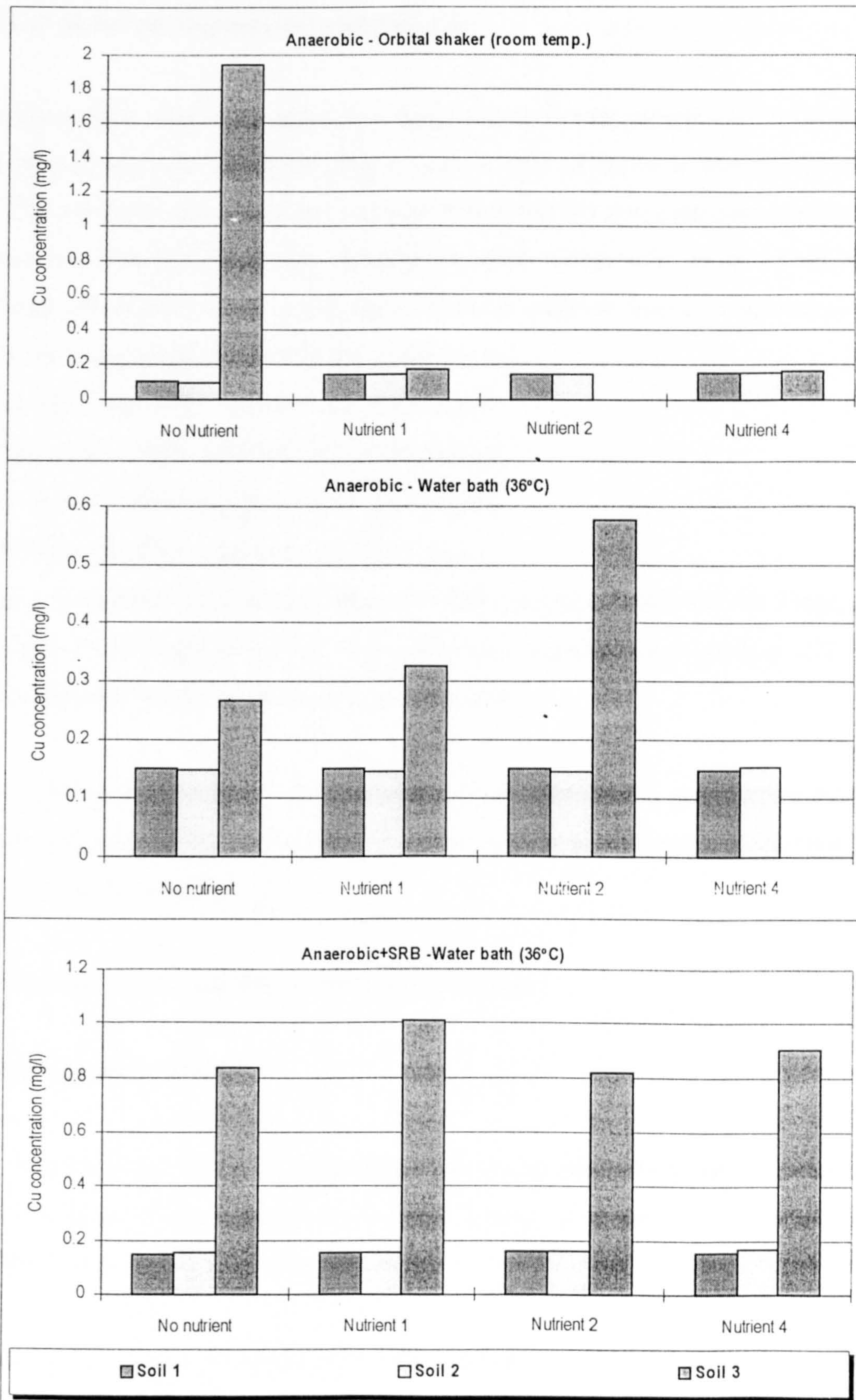


Figure 7. 11 Supernatant copper concentration – anaerobic slurry test (Method II)

7.5 Soil Slurry in Respirometer (Method III)

Soil slurry tests were also carried out using a respirometer system and involved both aerobic and anaerobic conditions. There were 4 types of nutrients investigated namely N1, N2, N4 (that were similar to anaerobic tests), and N3 that contained a phosphorus source only. The experiment also included the addition of glucose as the carbon source and soil pH adjustment by acidification. Nutrient addition was investigated at three levels, as was glucose. Nutrient levels added were:

- at 0.5N1 (low), N1 (medium) and 2N1 (high);
- 2N2 (low), 10N2 (medium), and 20 N2 (high);
- 5N3 (low), 10N3 (medium), and 20 N3 (high);
- 5N4 (low), 10N4 (medium), and 20N4 (high).

There were 3 levels of glucose applied: G1 (2500 µg/g dried soil), G2 (37.5 mg/g dried soil), and G3 (75 mg/g dried soil). The code used is self explanatory such as 2N2 meant double strength of N2; G3 meant glucose at Level 3.

The respirometric soil slurry test involved determination of the gas evolved from the system, pH, gas analysis, and sulphate, zinc and manganese concentrations. Soils 4 and 5 were used in this method.

7.5.1 Results of the soil slurry respirometric tests.

i. Gas evolution results

The main advantage of using a manometric system is its capability of measuring small gas volume changes. The apparatus used was a Warburg respirometer which was operated manually using a constant volume manometer.

a. Comparison of gas evolution with different nutrient addition

Figures 7.12-7.19 show the gas production from both aerobic and anaerobic systems at three levels of each nutrient addition. Both Soils 4 and 5 exhibited different patterns of cumulative gas evolved from each set. The level of nutrients also showed different

patterns. The results of gas evolution comparisons are summarised in Table 7.1.

Comparing conditions with and without nutrient in the aerobic system showed that in Soil 5, all nutrient types except N2 resulted in higher gas evolution than those without nutrient addition. In Soil 4, N2 and N4 addition evolved significantly lower gas production than without nutrients. For anaerobic treatment, addition of N1, N2, N3 to Soil 5 demonstrated a significantly greater gas evolution. Similar results were also found for Soil 4 with the further significance of lowest gas evolution after the addition of N4.

Table 7.1 Relative gas evolution according to nutrient levels

Soil samples	Nutrients	Aerobic condition	Anaerobic condition
Soil 4	N1	$N1 < 2N1 \cong 0.5N1$	$N1 < 0.5N1 < 2N1$
	N2	$10N2 < 2N2 < 20N2$	$10N2 < 20N2 < 2N2$
	N3	$10N3 \cong 20N3 < 5N3$	$20N3 < 10N3 < 5N3$
	N4	$10N4 < 20N4 < 5N4$	$10N4 < 20N4 < 5N4$
Soil 5	N1	$0.5N1 < N1 < 2N1$	$0.5N1 < N1 < 2N1$
	N2	$2N2 < 20N2 < 10N2$	$10N2 \cong 20N2 < 2N2$
	N3	$5N3 < 20N3 < 10N3$	$10N3 \cong 20N3 < 5N3$
	N4	$5N4 < 20N4 < 10N4$	$10N4 < 5N4 \cong 20N4$

The reaction flask was not facilitated with an alkali well to trap CO₂ gas, hence the volume change in the manometer was not represented total gas production rather than CO₂. In this study therefore, some reaction flasks produced negative gas evolution values. The gas in the headspace was controlled by partial gas pressure. If the gas was not evolved, it should be consumed by the microbial mechanisms in the soil slurry. The amount of CO₂ evolved that represented microbial activity was measured by the volume of gas withdrawal at the end of the shaking period and the composition determined from analysis in the GC. The CO₂ results of Soil 4 (with pH around 11-12) and Soil 5 (pH around 8) could not be compared at different pH due to the lack of data on the bicarbonate concentration, hence, carbon dioxide concentration, in solution.

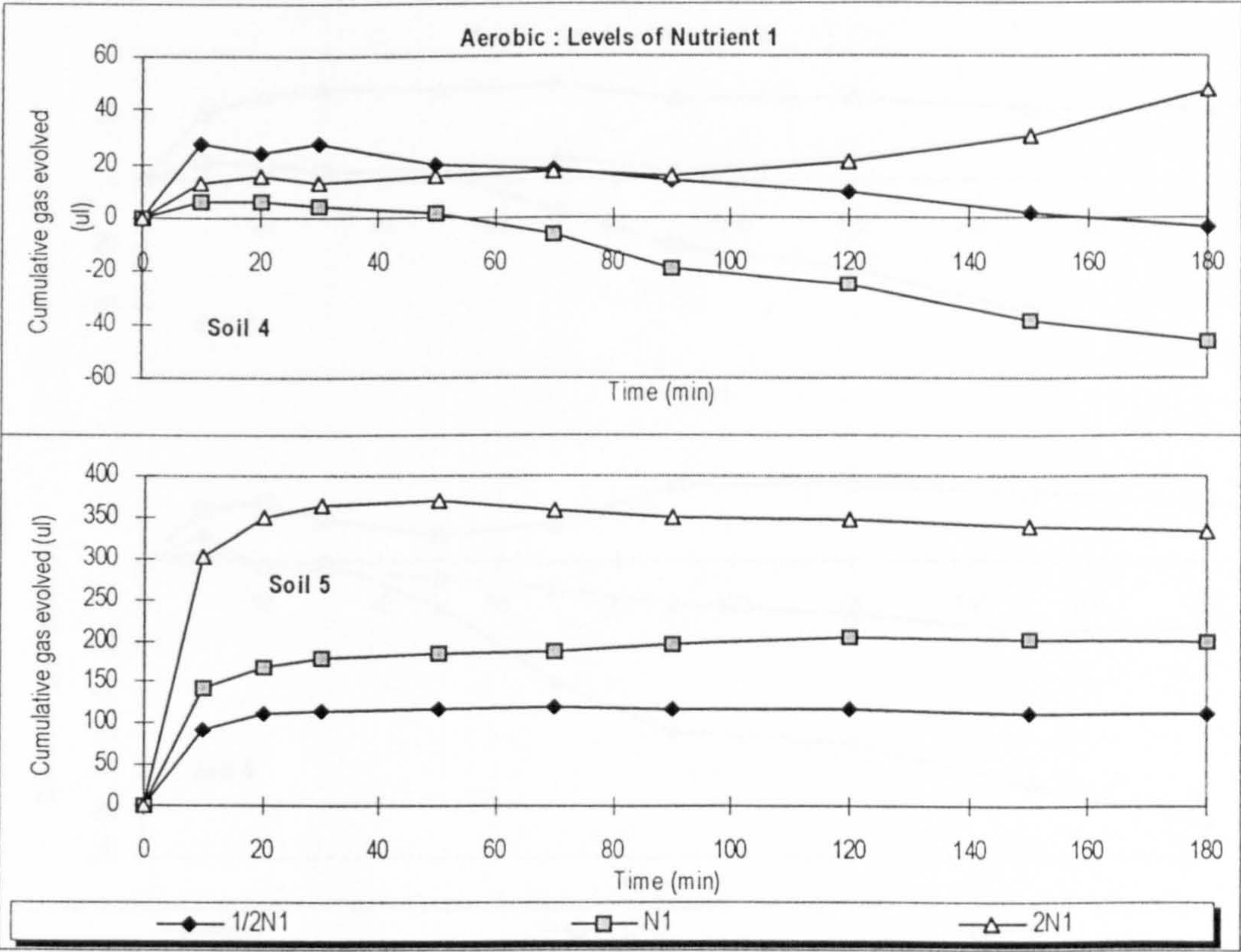


Figure 7.12 Gas evolution from aerobic process at 3 levels of Nutrient 1

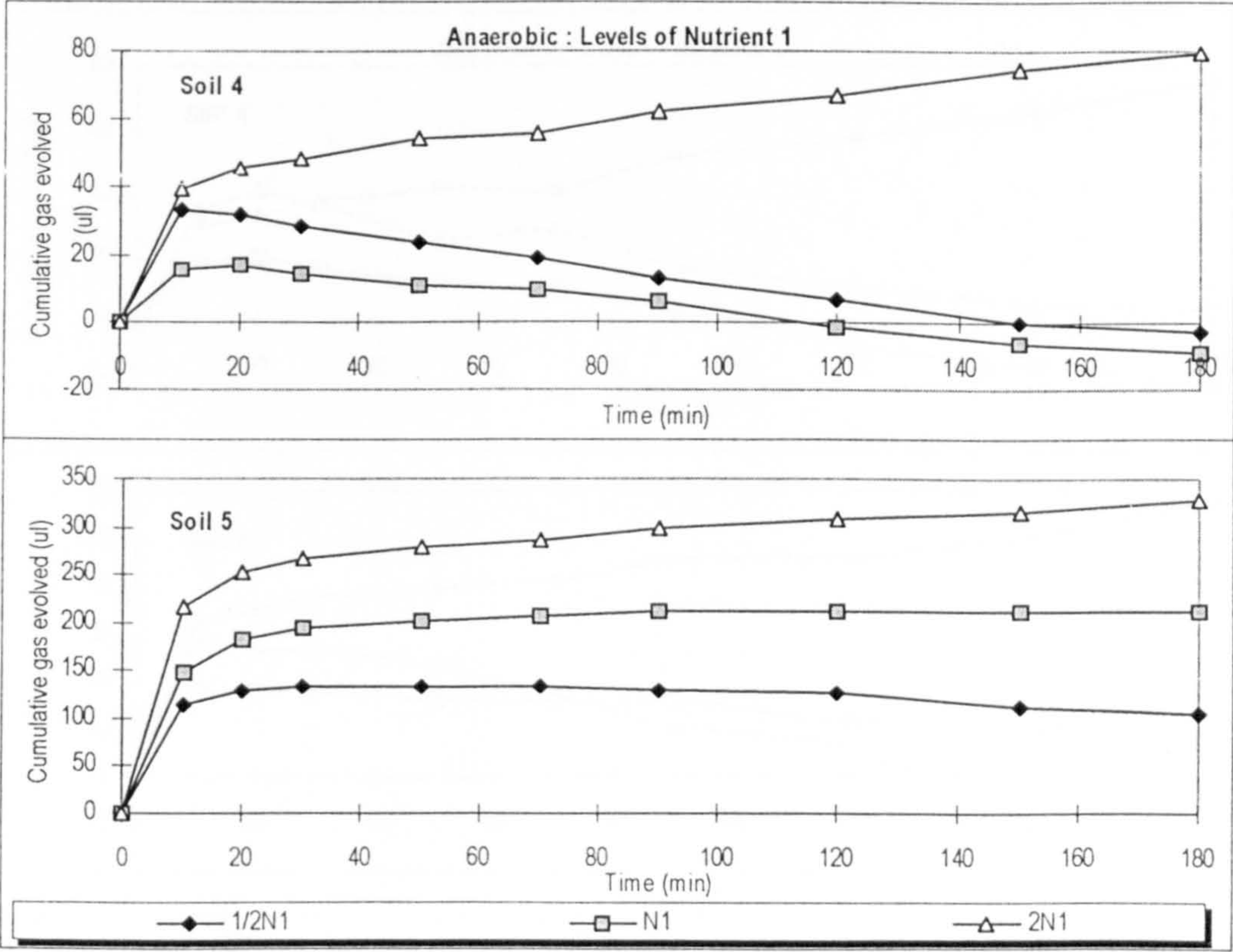


Figure 7.13 Gas evolution from anaerobic process at 3 levels of Nutrient 1

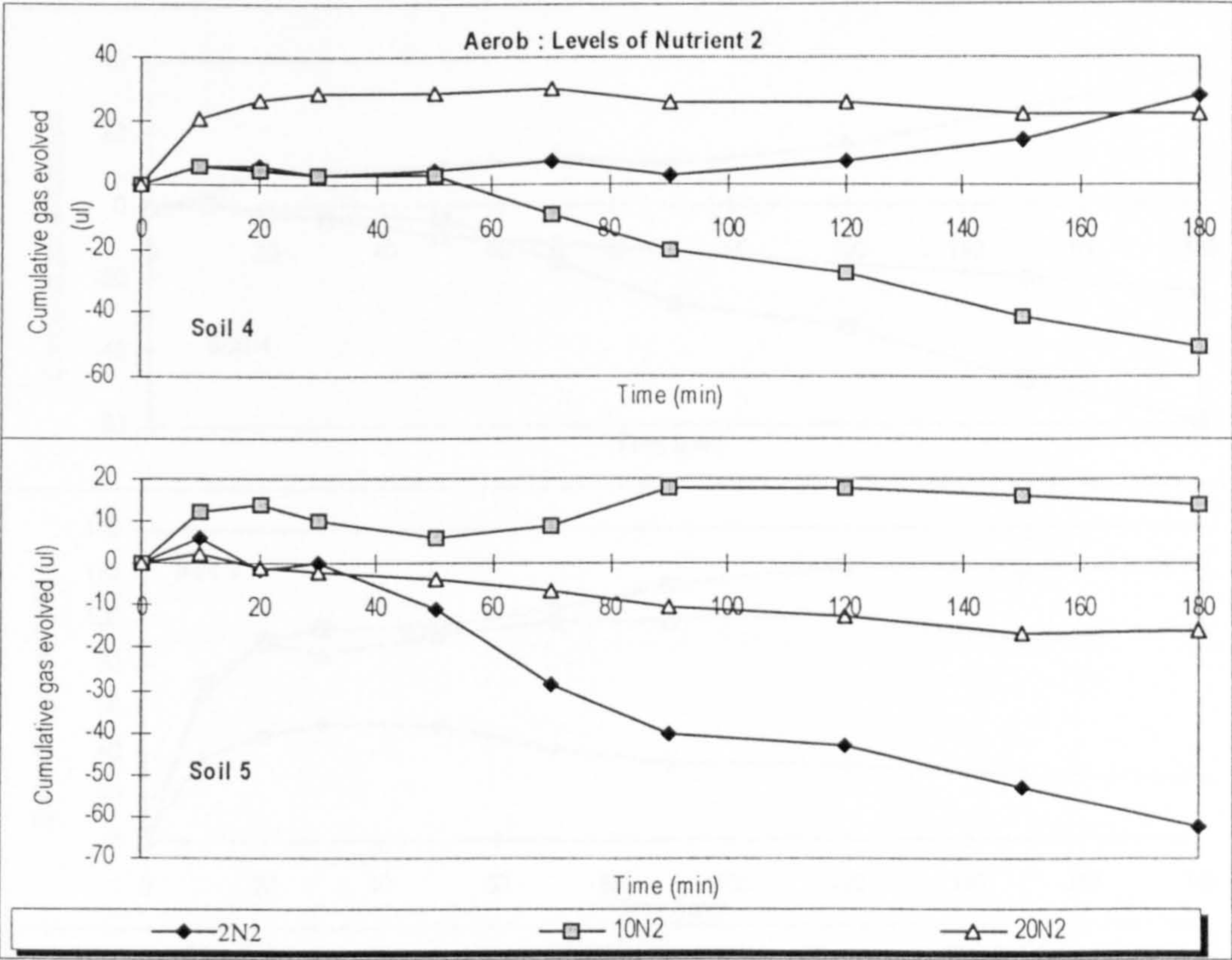


Figure 7.14 Gas evolution from aerobic process at 3 levels of Nutrient 2

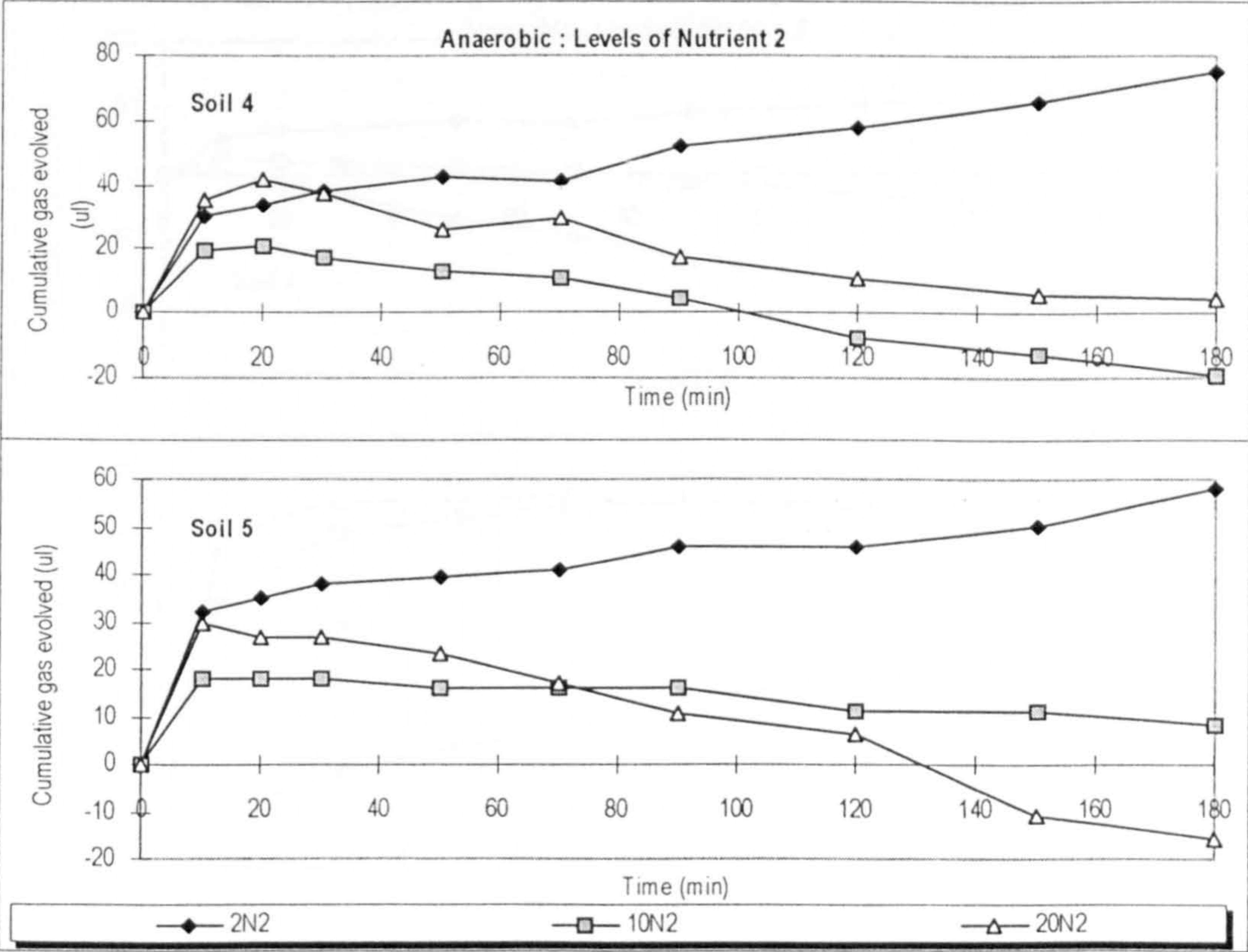


Figure 7.15 Gas evolution from anaerobic process at 3 levels of Nutrient 2

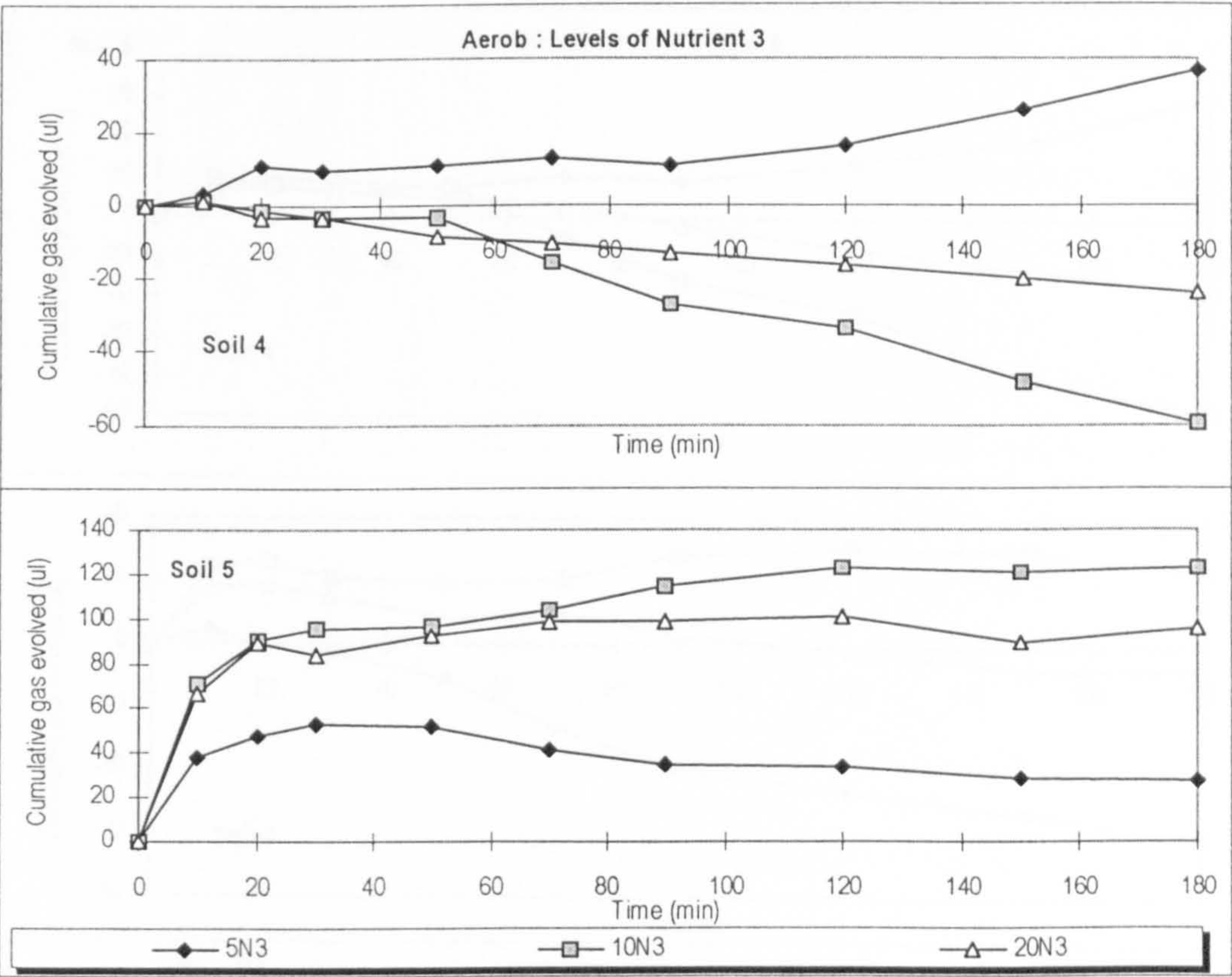


Figure 7.16 Gas evolution from aerobic process at 3 levels of Nutrient 3

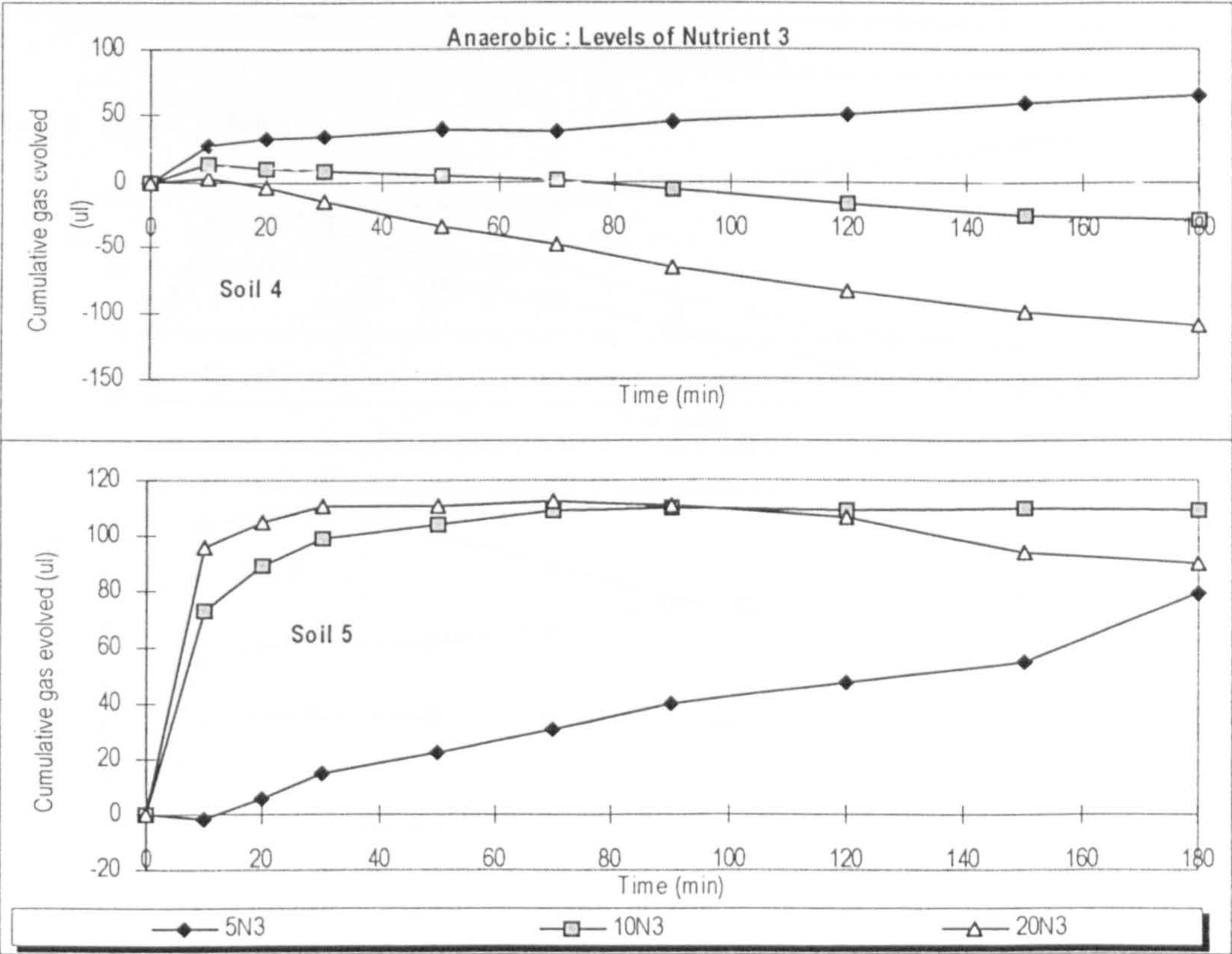


Figure 7.17 Gas evolution from anaerobic process at 3 levels of Nutrient 3

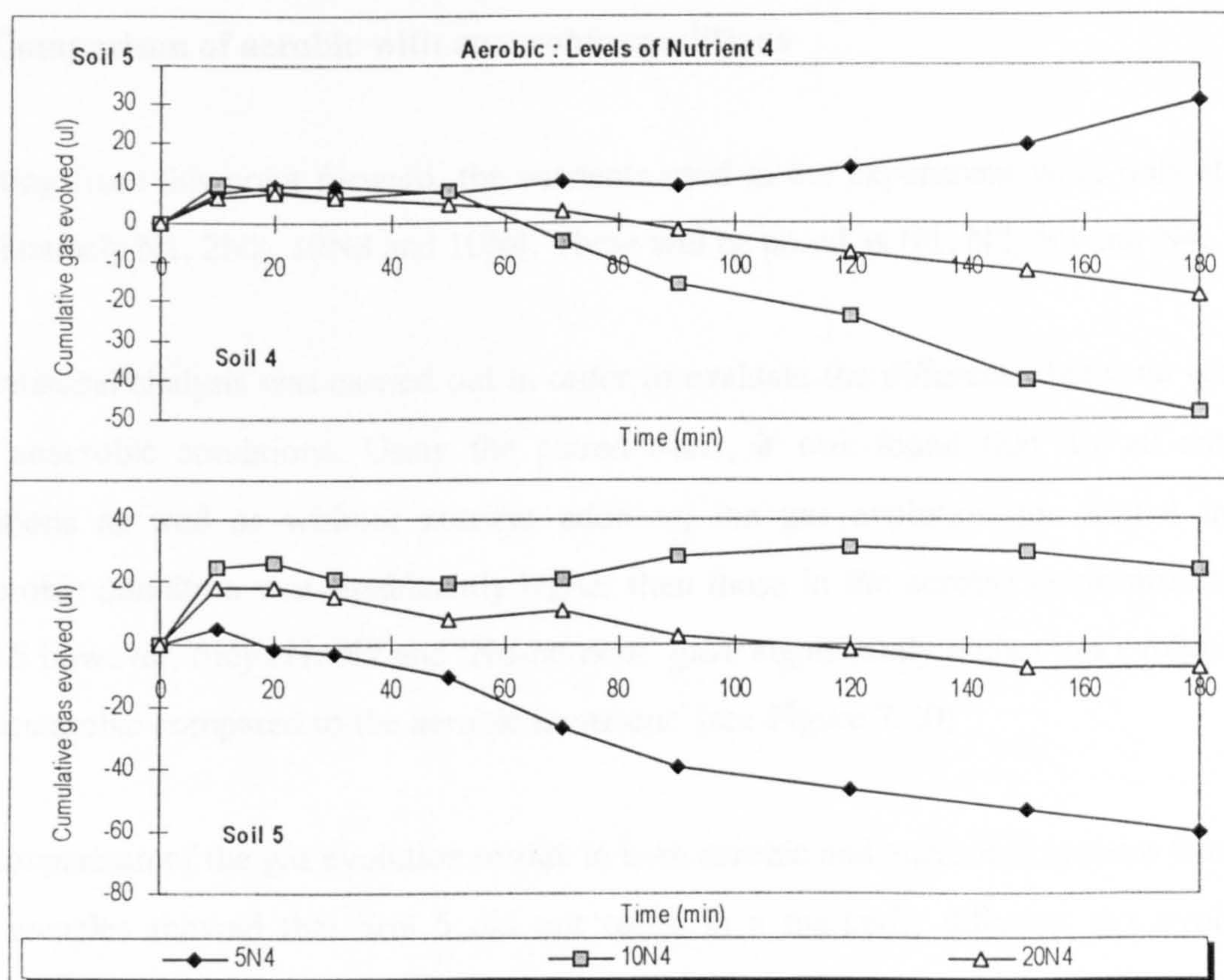


Figure 7.18 Gas evolution from aerobic process at 3 levels of Nutrient 4

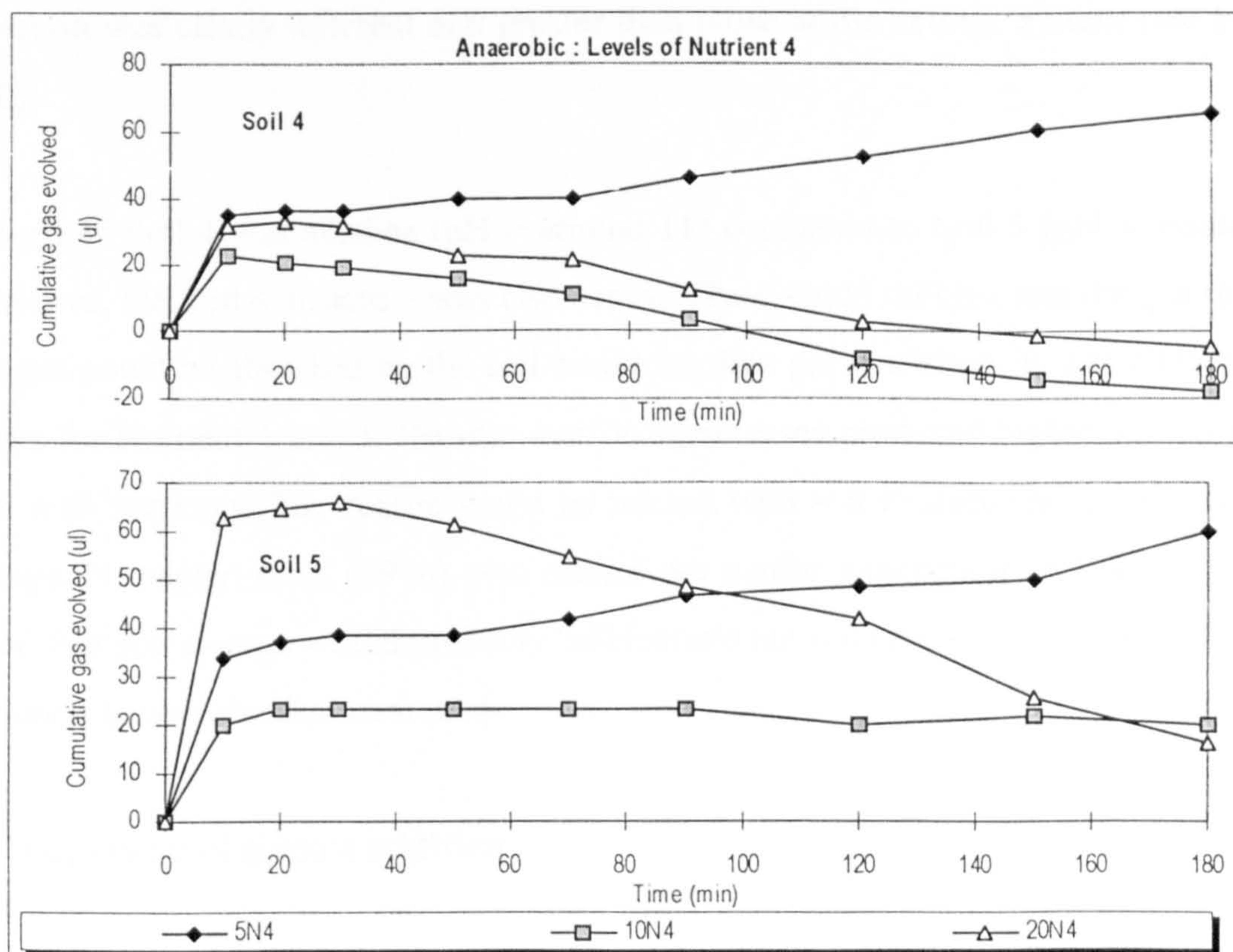


Figure 7.19 Gas evolution from anaerobic process at 3 levels of Nutrient 4

b. Comparison of aerobic with anaerobic conditions

Starting from this point forward, the nutrients used in the experiment were only at one level namely N1, 2N2, 10N3 and 10N4. These will be noted as N1, N2, N3 and N4.

A statistical analysis was carried out in order to evaluate the difference between aerobic and anaerobic conditions. Using the paired t-test, it was found that for all nutrient additions as well as without nutrient addition, the gas evolution for Soil 4 in the anaerobic condition was significantly higher than those in the aerobic environment. For Soil 5 however, only N1, N2 and 'No-nutrient' gave significantly higher gas evolution in the anaerobic compared to the aerobic treatment (see Figure 7.20)

A comparison of the gas evolution results in both aerobic and anaerobic systems from the soil samples showed that Soil 5 did not result in a markedly different gas evolution pattern. Nevertheless, the anaerobic system did differ and gave a significantly higher quantity of gas ($\alpha=0.05$) compared to the aerobic system. In Soil 4, the pattern of gas evolution was clearly different and greater than those in the aerobic system (see Figure 7.20).

The pH of Soil 4 was alkaline (pH = around 11) compared to Soil 5 (pH = around 8). Moreover, the Soil 4 structure was also sandy. These could differentiate the gas results. The gas could be absorbed by the soil producing less gas evolution. It was significantly shown for Nutrient 1 and 3. 'No-nutrient' in some cases produced higher gas evolution than with nutrients. The reason could be related with soil characteristics and also soil storage. Nannipieri et al. (1978) who carried out similar experiment with longer period noted that soil storage would probably differentiate the time required for maximum CO₂ evolution in two identical treatments.

c. Comparison of glucose addition

The investigation into the effect of glucose addition was only carried out in an anaerobic system. Glucose addition to Soil 5 resulted in a higher gas evolution after N2, N3, N4

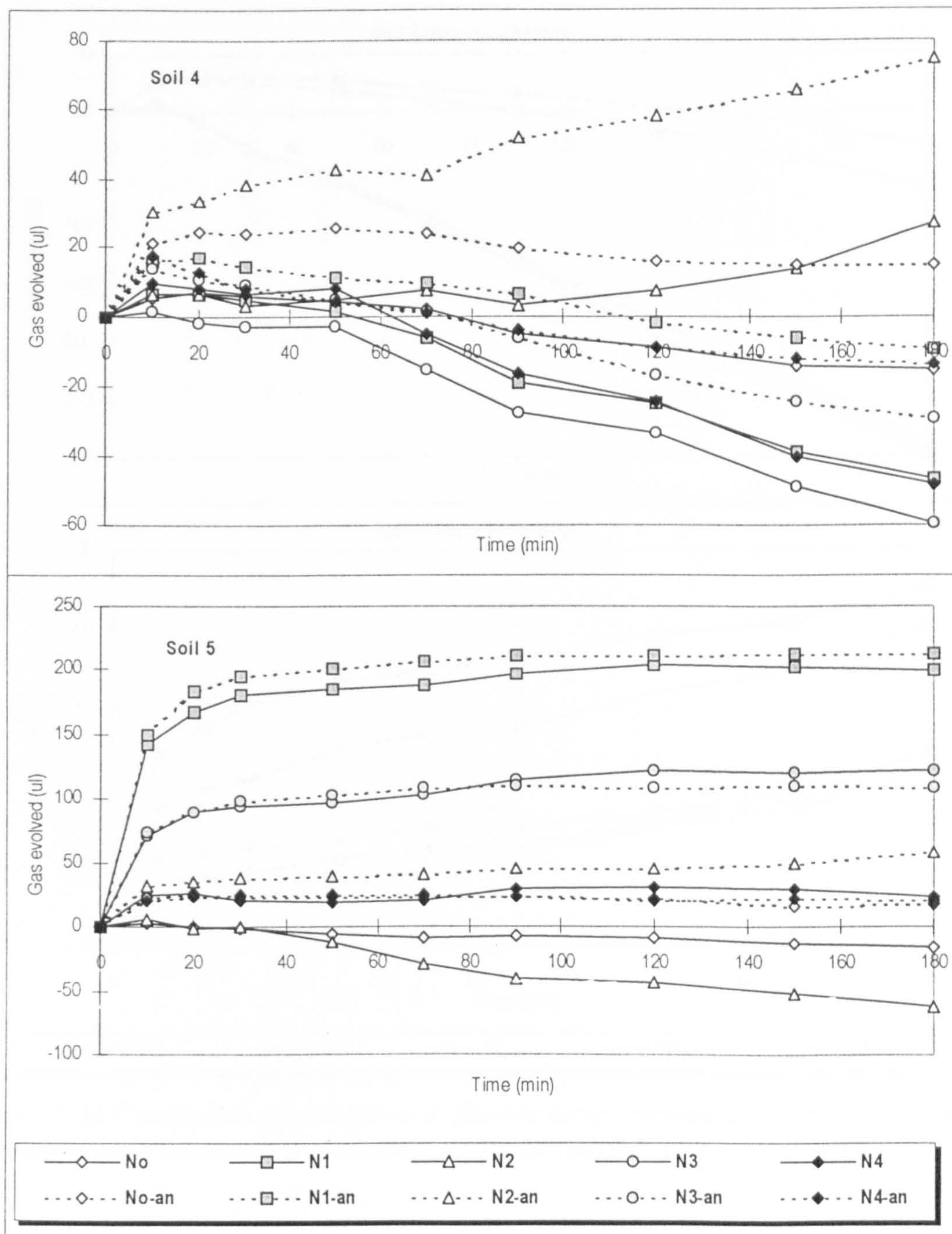


Figure 7.20 Cumulative gas evolution from aerobic and anaerobic respirometer conditions (No='No-nutrient' aerobic; N1=N1 aerobic; N2=2N2 aerobic; N3=10N3 aerobic; N4=10N4 aerobic; No-an='No-nutrient' anaerobic; N1-an=N1 anaerobic; N2-an=N2 anaerobic; N3-an=10N3 anaerobic; N4-an=10N4 anaerobic)

and even for 'No-nutrient' added than those without glucose addition. In Soil 4, different results were obtained. The addition of glucose and N3 was the only condition which gave a higher gas evolution than those samples without glucose addition. Glucose plus N2, N4 and 'No-nutrient' produced significantly lower gas. Gas evolution with glucose plus N1 addition showed no difference compared to that without glucose addition.

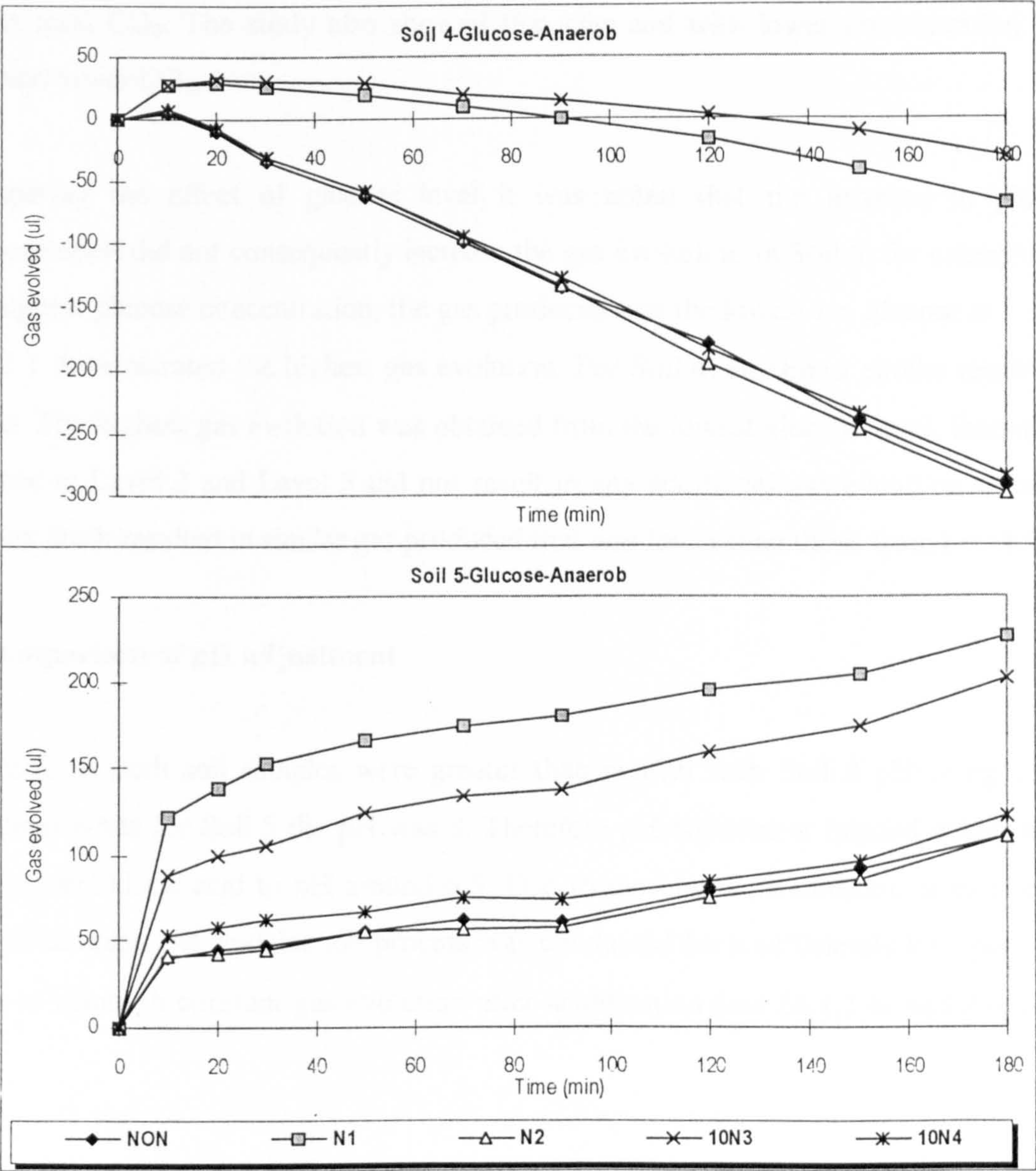


Figure 7.21 Cumulative gas evolution in glucose supplemented anaerobic respirometer conditions (Non='No-nutrient'; N1=N1 nutrient; N2=N2 nutrient; 10N3=10N3 nutrient; 10N4=10N4 nutrient)

Comparing the effects of nutrient types and glucose addition, in Soil 5 the gas evolution for N2 was the lowest, followed by 'No-nutrient' addition, N4, N3 while the highest was with N1 addition. In Soil 4 the effect of nutrient was different. 'No-nutrient', N2, N4 addition were similar in the amount of gas evolved and also the lowest, followed by N1 while the highest resulted from N3 addition (see Figure 7.21)

Nannipieri et al. (1978) also found that soil with glucose and N & P addition had higher CO₂ evolution rate in the first 24 hrs than soil without P. After 82 hrs, both showed equal evolution. They also noted, increase P enhanced initial CO₂-C evolution but did not

affect total CO₂. The study also showed that clay soil with lower concentration of P evolved lower CO₂.

Comparing the effect of glucose level it was noted that the increase in glucose concentration did not consequently increase the gas evolution. In Soil 5, for example, for the highest glucose concentration, the gas produced was the lowest i.e. glucose at Level 1 demonstrated the highest gas evolution. For Soil 4, an almost similar result was found. The highest gas evolution was obtained from the lowest glucose level. Increase in glucose at Level 2 and Level 3 did not result in any additional gas evolution from the system. Both resulted in similar gas produced that was lower than those from Level 1.

d. Comparison of pH adjustment

The pHs of both soil samples were greater than neutral with Soil 4 pH being 11-12 (alkaline) while for Soil 5 the pH was 8. Therefore pH adjustment implied acidification using hydrochloric acid to pH around 6.5. Due to the high concentration of calcium in the soil samples, the acidification process was conducted for a sufficiently long period in order to establish constant gas evolution after acidification (see §6.1.1-iii and Appendix C).

As shown in Figures 7.21 and 7.22, for Soil 5, gas evolution from treatment with acidified soil samples plus N2, N4 and 'No-nutrient' added was significantly lower than for those from the unacidified sample. In the case of N1 and N3 addition, however, the gas evolved from the acidified sample was found to be greater than those from the unacidified sample.

Evaluation of the effect of nutrient addition in acidified Soil sample 5 showed that in all cases, nutrient addition produced more gas than without nutrient addition. Nutrient 1 generated the highest gas evolution, followed by N3, then N4, with N2 being the lowest amount. For Soil 4, the results were different whereby only N1 and N3 addition gave a greater quantity of gas than the sample without nutrient. Results for the addition of N2 and N4 did not significantly differ from the sample without nutrient addition.

The effect of glucose level in acidified soil samples was also investigated. Both acidified soil samples gave similar results. Glucose Level 3 in the acidified samples statistically showed the greatest amount of gas compared to those at glucose Levels 1 and 2. These latter two levels generated no significantly different gas evolution at (see Figure 7.23)

ii. Changes in pH

Measurement of pH of the supernatants from the Warburg flasks was carried out after the shaking period was completed. According to an ANOVA analysis on both soils, it was found that the pH was significantly affected neither by nutrient type nor nutrient levels in both aerobic and anaerobic condition.

Although the pH of the soil slurry was not affected by either nutrient type or levels, addition of glucose and soil acidification altered the pH after the shaking period. In Soil 5 the pH after glucose addition alone changed according to the nutrient types. N1 and N3 did not affect the pH whereas for N2 and N4, the pH was increased by around 1-2.5 units. For Soil 4, glucose had an almost similar response and differed by around 0.5 unit. For Soil 5 in which acidification did not reduce the pH significantly, the pH response was found to be noticeable. Nutrient 3 addition resulted in the pH to decrease to a lower value than for other nutrients after glucose addition and acidification. A similar result was also obtained for Soil 4.

Statistically, with glucose and acidification the pH of the slurry of Soil 5 was affected neither by the treatment condition nor the nutrient types. In the case of Soil 4, the pH was significantly influenced by both the treatment condition and the nutrient types. This could be due to the significant effect of pH changes in the acidification process for Soil 4.

iii. Changes in sulphate concentration

Under aerobic conditions, nutrient levels were found to significantly affect the sulphate concentration in the Soil 5 tests and had a greater impact than levels in Soil 4 tests. Nutrient level 1 (the lowest) led to the release of lower sulphate concentrations in Soil 5.

In Soil 4, N3 addition released the highest sulphate concentration compared to other nutrient levels. Anaerobically, only in Soil 4, did the nutrient types show statistically significant different sulphate response. However, the results for nutrient types were similar to those under aerobic conditions. N3 addition gave the highest sulphate concentration release.

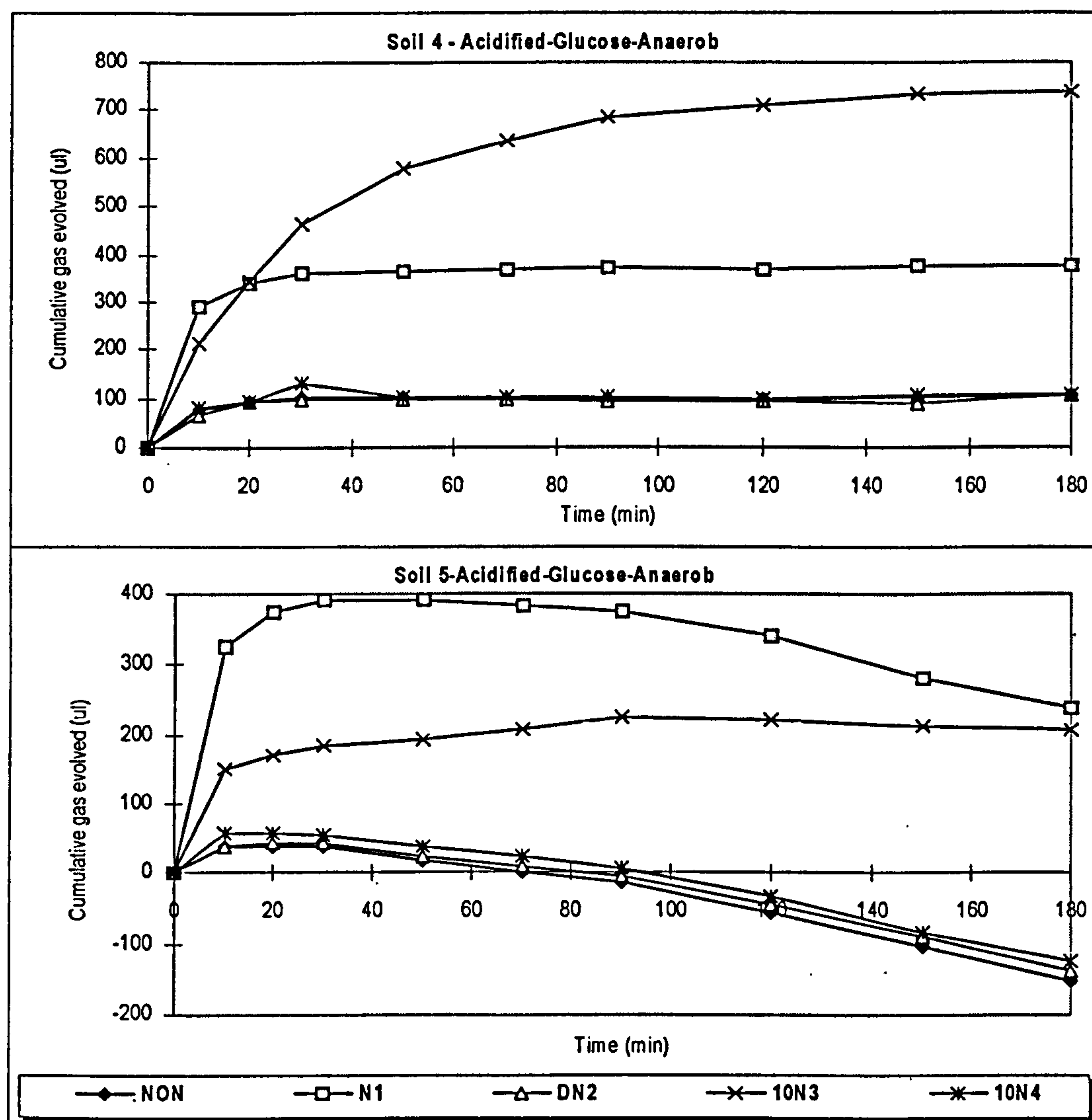


Figure 7.22 Gas evolution in acidified soil - glucose- anaerobic respirometer condition (Non='No-nutrient'; N1=N1 nutrient; DN2=2N2 nutrient; 10N3=10N3 nutrient; 10N4=10N4 nutrient)

Comparing the results of adding glucose and acidification adjustment, it was noted that the sulphate releases from both Soil 4 and Soil 5 were slightly lower if there was no nutrient added with the glucose. However, for Soil 4, the addition of N3 plus glucose generated a ten times greater sulphate concentration than with other nutrients. Acidification of Soil 4 released more sulphate into solution.

For Soil 5, glucose addition alone (without acidification) in the anaerobic system increased the sulphate in the supernatant. The higher the quantity of glucose added, the

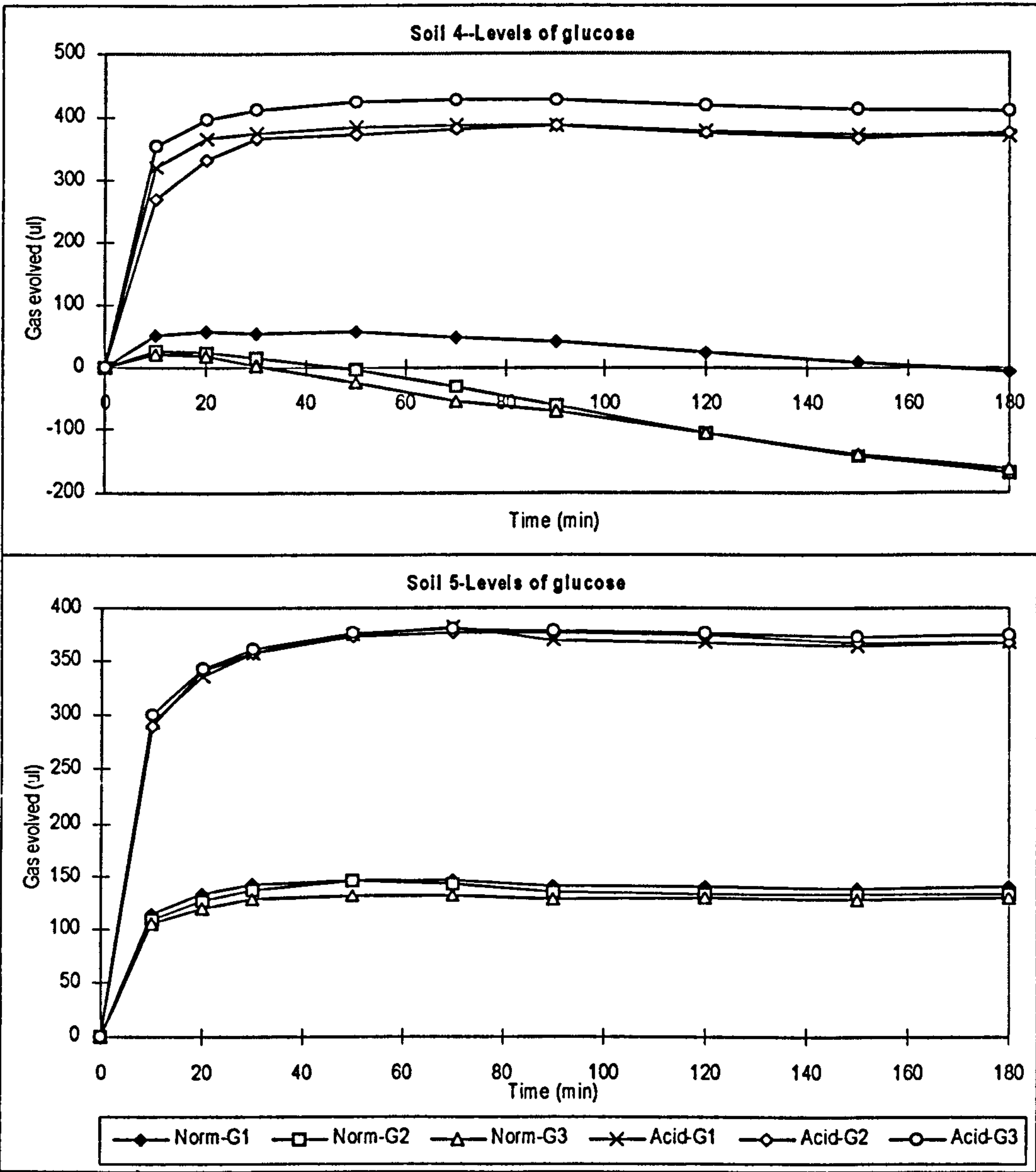


Figure 7.23 Cumulative gas evolution in 3 levels of glucose-anaerobic respirometer condition (Norm= Normal soil condition; Acid=Acidified soil; G1=Glucose Level 1; G2=Glucose Level 2; G3=Glucose Level 3)

more sulphate was released. In the case of acidification and glucose addition at the same time, sulphate did not significantly increase. This implied that an increase in glucose levels alone resulted in oxidation of the sulphur instead of immobilization.

The Soil 4 result was not identical. An increase in glucose addition either alone or accompanied by acidification did not change the amount of sulphate released into solution.

iv. Results of zinc and manganese concentrations

The zinc releases were affected by nutrient types for both aerobic and anaerobic processes in Soil 5. Zinc was found at its lowest concentration after N1 addition. N3 in contrast liberated the highest concentration in the Soil 5 tests. These results were seen to be similar for both aerobic and anaerobic conditions. Manganese on the other hand, in Soil 4, was more affected by the levels of nutrient. Medium levels of nutrient released lower Mn concentration under aerobic condition. Mn in Soil 5 was significantly affected by nutrient types in anaerobic systems with N1 and N3 addition producing lower Mn concentrations.

The effect of glucose addition on zinc release demonstrated that similar results were produced for anaerobic only and anaerobic plus glucose addition. The addition of N1 showed a slightly lower Zn concentration. N3 on the contrary was found to increase Zn release into the supernatants for both soil samples. This was more pronounced in acidified samples where N1 addition produced a much lower Zn concentration than other nutrient types.

When increasing the glucose level in the system, Zn was found at the lowest concentration for the lowest glucose level for Soil 4. In acidified soils the glucose level increase did not change the level of Zn mineralization. This was also found to be the case for Soil 5 which showed almost similar Zn releases for both glucose alone and for acidified samples.

Manganese was found to be at the lower concentration after N1 addition. Glucose addition and soil acidification did not affect the release of Mn into solution and moreover an increase in the glucose added released almost the same amount of Mn into the supernatants.

Statistical analyses showed that Zn and Mn in Soil 5 were affected by nutrient types and treatment by anaerobic, anaerobic+glucose, and anaerobic+acidification+glucose. However, for Soil 4, Zn and Mn were only altered by nutrient types.

v. CO₂ evolution

Besides gas production, which was measured in the respirometer, an attempt was made to measure the concentration of CO₂ in the gas. This procedure, however, was not simple since gas sampling from the small flask influenced the setting of the manometer. One millilitre gas sample removal was sometimes too large to be accommodated in the headspace of the flask up to the final level of the manometer fluid, some of which spilled into the soil mixture. The CO₂ produced however, was a useful parameter in respiration studies. CO₂ in the gas sample was measured by Gas Chromatography.

During the experiment the GC was sometimes not able to detect small peaks in the results. Therefore, the CO₂ produced was sometimes represented as a percentage (for detectable peaks), and sometimes noted as positive or negative according to the height of the peaks. Experimental comparisons could only be made between samples releasing CO₂ in detectable amounts. This was demonstrated only for Soil 5 which exhibited greater CO₂ production than Soil 4.

For Soil 5 under aerobic conditions, it was noted that only N1 and N3 addition produced CO₂ at around 0.55% to 1.4% and 0.41% to 0.55% respectively. An increase in N1 concentration increased the CO₂ evolved. For anaerobic conditions the percentage of CO₂ in the gas evolved was slightly lower at 0.44% to 1.2% and 0.4% to 0.57% for N1 and N3 nutrient respectively. Gas evolved from Soil 4 on the contrary, did not show any detectable CO₂. Nutrients 1 and 3 were considered to be the most appropriate for soil samples as they always gave a higher response than other nutrients.

Larger reaction flask could be used to increase the gas amount detected. However, a trial with 100ml flask (instead of 50ml) was not satisfactory as the water in the water bath splashed and the movement of manometer was disturbed.

Glucose addition to the system for Soil 4 however resulted in a small CO₂ production after N3 addition. Acidified samples together with glucose addition markedly increased the CO₂ in both samples. All nutrient additions showed a release of CO₂ in Soil 5. The highest was found for N3, followed by N1 and a much lower amount after N2 and N4 addition. For Soil 4, the level of CO₂ detected after acidification was significantly higher than those for Soil 5. Nutrient 3 addition resulted in the highest level (4.38%), for N1 it was 1.36%, for N2 it was 1.13% and N4 0.7%. Furthermore, CO₂ was also released under 'No-nutrient' condition at 0.37%. This was evidence that the acidification process for Soil 4 released CO₂ due to chemical reaction. As explained by Holderness and Lambert (1966), reaction of hydrochloric acid (as the neutralizing agent) with chalk or CaCO₃ produced CO₂.

Increase in glucose levels to the system for Soil 5 showed that for the unacidified soil, an increase in the glucose added did not increase CO₂ production. Glucose at Level 1 (2500µg/l) resulted in more CO₂ than from higher glucose levels. In contrast, CO₂ evolution from acidified Soil 5 increased as the glucose levels increased.

7.5.2 Summary of respirometer results

Respirometry resulted in a more accurate detection of gas produced from the system. Aerobic and anaerobic conditions could also be compared. Soils 4 and 5, which were different in structure and composition, resulted in different patterns of gas evolution. Anaerobic conditions exhibited slightly greater gas evolution than aerobic conditions. The types of nutrient added influenced the gas production, sample pH and the release of Zn and Mn. Changes in treatment such as glucose addition and acidification of the soil samples also affected the parameter of interest. Glucose addition at higher concentrations did not significantly increase the gas evolution while acidification of the soil sample resulted in more gas than unacidified samples. Moreover, for acidified soil samples increased glucose concentration resulted in markedly higher gas production.

The pH in Soil 5 tests was not significantly affected by treatment or nutrient types. In contrast, the pH in the acidified Soil 4 was significantly altered by treatment and nutrient types. The pH decreased in both soils after N3 addition to acidified samples, but on the

other hand the increased glucose level did not alter the pH.

Under aerobic conditions, Soil 5 supplemented with N1 showed lower sulphate release than with other nutrients whereas Soil 4 released more sulphate after N3 addition. In anaerobic acidified soils, for both Soils 4 and 5, glucose only addition resulted in lower sulphate mineralization, however, glucose plus N3 addition to Soil 4 leached a 10 times greater sulphate concentration.

As the glucose level increased the sulphate concentration was found to be greater in unacidified Soil 5. The sulphate concentration in Soil 4, on the contrary, did not change after increases in glucose and acidification.

Zn was found in lower concentrations after N1 addition and higher after N3 addition to Soil 5. Glucose addition to the system with acidification plus N1 addition released lower Zn concentration. However, glucose seemed to be more effective at the lowest concentration (2500µg/l). Manganese was also found to be at lower concentration after N1 addition. Glucose and acidification, however, did not affect the Mn concentration.

Comparing the results of CO₂ production, it was noted that Soil 5 always evolved more CO₂ after N1 addition whereas Soil 4 was more affected by N3 addition. Glucose increases did not increase the CO₂ production in unacidified Soil 5. The CO₂ evolved was higher when the soil was acidified and the glucose level was increased. This was clearly demonstrated in acidified Soil 4.

7.6 Discussion

Soil slurry reactors were usually preferred as they were simple to manage and control. It also enhanced microbial reactions so that the time required was shorter. Method I, which was used in this study, was operated as an SBR (sequencing batch reactor) system as suggested by Irvine et al (1993). Treatability reactors with similar principles with regard to CO₂ evolution measurement by alkali traps have been used by many researchers (Rogers et al., 1993; Govind et al., 1994). However, this study recognised that the CO₂ evolution measurement was not sensitive enough to compare treatment types between

soil samples. This could be due either to the low level of nutrients added or to the low level of activated sludge applied. Sharabi and Bartha (1993) suggested adding 0.5 or 1.0 mg of test compound/ g soil to obtain results that were not interfered by CO₂ evolution from the soil background. On the other hand, the method used in this study was able to detect pH differences and also VSS measurement was possible in the experiments. This evidence suggested that the equipment in Method I was more suitable for aerobic treatability tests with some improvement in equipment arrangement. This includes continuous control of aeration rate, alkali traps for air supply to avoid CO₂ input, control of agitation rate, more accurate titration measurement and continuous pH measurement.

Soil slurry experiments such as in the anaerobic slurry test (Method II) was developed by Bartha and Pramer in the 1960s. The same principles and apparatus are still in use. The method has proved to be reliable and accurate (Sharabi and Bartha, 1993). Triplicate biometers for long period experiments exhibited 5% standard deviation and for shorter periods the standard deviation was only 2%. The standard deviation in this study ranged from 1.2% in 'room temperature (Setting 1)'; 3.2% in 'water bath 36°C (Setting 2)'; and 38.5% in 'water bath+SRB (Setting 3)' Setting 3 showed this very high deviation originating from Soil 1 and Soil 3 performances. Standard deviation in Soil 2 was 4.04% and still in accordance with results obtained by Sharabi & Bartha (1993). The reason for this could be related more to the soil sample, experimental sampling and SRB conditions rather than the biometer design.

Some modifications that were applied however, in the biometer used in this study, could possibly slightly reduce the performance. One modification, for example, included use of Carbosorb instead of Ascarite (which was more expensive) as CO₂ traps in the flask. Another was the procedure of the experiment itself which could not accommodate pH measurement without opening the flask. However, the study did show some important results from the anaerobic system applied in the tests such as the effect of temperature. The results were in agreement with Belkin et al. (1985) who found that sulphate respiration is favoured at high temperatures. The biometer design and procedure could be improved, for example by an additional port for slurry sampling and pH measurement.

Respirometry has been used for treatability tests for many years, over which time the apparatus has developed from manually operated to automatic, sophisticated operation. Columbus Ltd (in the USA) for example designed an automatic respirometer known as the Micro/Oxymax which is capable of measuring gas changes and some organic chemical parameters of microbial metabolism. The Warburg system was the first apparatus of its kind and the operation is still manual. However, with some modifications, the Warburg respirometer is able to produce accurate gas measurement under anaerobic conditions. This manometric apparatus is able to detect minimum gas volume changes of approximately 0.03 μ l. This was one valuable advantage of the application of respirometric test (Method III). The time required was also shorter than other methods that were operated as reactors. In addition, CO₂ could be measured by GC without a trap inside the flask. Moreover, Cleve et al. (1979) who compared four methods of respiration measurement claimed that measurement of CO₂ evolution from respiration was more accurate by titration or infra red gas analysis. Other methods they compared were gas chromatography and the Gilson respirometer which showed lower results while infra red gas analysis performed better with KOH absorption.

It is more appropriate to measure CO₂ evolution than O₂ uptake during metabolism. In the manometer for O₂ uptake determination, there are gases other than CO₂ which could interfere with the manometer level. Moreover, O₂ uptake in the soil study required the soil environment to be completely aerobic. In soil samples it is likely that O₂ uptake underestimated the level of activity. CO₂ measurement also encountered problems from non-biological production of CO₂ through chemical decarboxylation, cell-free enzymes or from free carbonates in the soil (Stotszky in Dennis, 1971). This was also considered in this study as the soil sample contained a high level of calcium carbonate which generated high CO₂ after acidification of the soil samples.

To obtain reproducible results, the procedure in respirometry (Method III) included soil slurry homogenisation by shaking the mixture for 6 hours prior to pre-treatment. This was supposed to avoid gas fluctuations at the beginning of the shaking period which was experienced in initial trials. The results of gas production from 'No nutrient' amendment were compared and taken into the calculation as a correction factor.

Tabulated summaries of soil slurry results are in the following Tables 7.2 - 7.4. In theory sulphate and heavy metals reduction in the soil studied should also show decrease in pH and increase in microbial activity responsible for the process. Although different soil samples were investigated in three methods used but Soil 1 and 2 (both were contaminated soil) were similar in structure and types with Soil 5. Therefore, their results were likely to be similar.

Aerobic slurry test showed that flasks without aeration contained more VSS and lower pH. Furthermore, the test noted the increase of nutrient added also increased VSS and lowered the pH. This supported the conclusion that the more appropriate process was without aeration.

Anaerobic slurry test resulted that at room temperature the pH and sulphate concentration was lower in Soil 1. This was in accordance with study by White and Gadd (1991) that dissimilatory sulphate reduction was optimum at 20° C. The test also indicated that Nutrient 1 released more CO₂ production. However Zn and Mn reduction were more likely required SRB addition.

Results from respirometry revealed that anaerobic condition produced more gas. Soil 4 with N3 and Soil 5 with N1 addition gave more gas than other nutrients. Gas evolution increased with glucose addition. Increases in added glucose level, however, did not necessarily increase gas production. Addition of glucose to acidified samples resulted in greater gas production. This was seen in the CO₂ results from both soil samples. Unfortunately, glucose addition did not change the pH. Acidification process was more responsible for reducing the pH.

Acidification changed sulphate concentration. In Soil 4, sulphate concentration was available at around ten times than that in an unacidified soil whereas for Soil 5, acidification slightly reduced the sulphate. Zinc concentration also increased markedly after acidification for both soils. Increase glucose level in Soil 5 also increased sulphate concentration whereas for Soil 4 an increase in glucose level did not alter the sulphate in solution.

After acidification and glucose addition, Soil 4 released detectable CO₂ evolution. The amount of CO₂ evolved was even higher than those from Soil 5. This could have resulted from CO₂ production from the inorganic source because Soil 4 was rich in CaSO₄. Acidification itself would release some CO₂ gas (Holderness and Lambert, 1966).

Table 7.2 Summary results of aerobic slurry test - Method I

Parameters	Results
pH	pH changed in different treatment/flasks; pH of control in most cases found to be higher than with nutrient addition; pH of unaerated was usually the lowest
CO ₂ evolution	CO ₂ evolution in unaerated flask was very low; CO ₂ measurement was not sufficiently sensitive
Sulphate concentration	Sulphate in Soil 2 appeared to be reduced by '5ml' N1 addition
Zinc concentration	Zinc could be decreased after '2.5ml' N1 addition
VSS content	For both Soils 1 and 2 increase in nutrient amount added increased the VSS content

Table 7.3 Summary results of anaerobic slurry tests - Method II

Parameters	Results
pH	pH was affected by treatment settings pH in flasks shaken in the 'water bath 36°C' was higher than that in the 'room temperature'
CO ₂ evolution	Results of CO ₂ measurement was not statistically significant but it could be compared between treatment conditions
Sulphate concentration	For Soil 1: sulphate was lower at Setting 1 (shaking at 'room temperature') For Soil 2: it was found in Setting 3 ('water bath 36°C+SRB sludge')
Zinc and Mn concentration	Zn and Mn concentration were found to be lower in Setting 3 ('water bath 36°C+SRB sludge')
Cu concentration	Cu was not affected by treatment condition or nutrient

Table 7.4 Summary results of slurry tests in respirometer - Method III

Parameters	Results
pH	pH was not affected by nutrient types or levels Glucose addition did not alter the pH Acidification of Soil 4 affected the changes of pH
CO ₂ evolution	Soil 5+N1 exhibited more CO ₂ evolution Soil 4+N3 slightly released more CO ₂ evolution Acidified samples +glucose gave higher CO ₂ produced
Gas evolution	Nutrient types and levels differentiated gas evolved; Anaerobic condition increased gas evolution from Soil 4 and Soil 5 after N1 and N3 addition; Glucose addition gave a higher gas evolution; Acidified sample increased gas evolution; Increase glucose levels in acidified samples increased gas production.
Sulphate concentration	Soil 5: with N1 resulted lower sulphate concentration; Acidified Soil 5 and glucose gave lower sulphate concentration; Increase in glucose amount added produced higher sulphate concentration; Soil 4: with N3 released more sulphate into solution; Acidified Soil 4+N3 produced 10 times higher sulphate; Glucose levels increased did not change sulphate concentration.
Zinc concentration	N1 addition appeared reducing Zn in the solution; N3 addition released more Zn in the solution; Acidified Soil 5+glucose+N1 decreased Zn released.
Mn concentration	N1 addition released lower Mn concentration; Glucose and acidification did not affect Mn concentration.

7.7 Conclusion

The soil slurry experiment was aimed to investigate the biological treatability of soil samples and appropriate environments including type of nutrients. It was suggested that Nutrient 1 was more appropriate to increase microbial activity as shown in the respirometry experiment. The ‘Without aeration’ condition showed higher VSS content and the anaerobic condition gave higher gas evolved. In Soil 5, N1 and N3 released CO₂ whereas in Soil 4, CO₂ was only detected when glucose was added. Addition of glucose significantly increased the gas evolved in Soil 4. The N1 was considered to be applied in the soil column as a second stage test. The type of nutrients would be again tested and confirmed using microbial growth parameters in soil extracts.

CHAPTER 8

RESULTS AND DISCUSSION OF AEROBIC MICROBIAL GROWTH EXPERIMENTS ON SOIL EXTRACTS

8.1 Background

These initial tests were intended to investigate the appropriate nutrients that would stimulate growth of the indigenous bacteria in a soil extract. The assessment would be made by measurement of turbidity after shaking the soil extract and nutrient mixtures. The nutrients used were similar to those in the slurry tests. The test also included an investigation of nutrient level, glucose addition and pH adjustment (acidification). An estimation of the microbial population was obtained from the results by correlation between absorbance and total bacterial count using the acridine orange method. Unfortunately, it was not possible to carry out the tests under anaerobic conditions, therefore comparisons between aerobic and anaerobic were not possible.

8.2 Establishment of Procedure

Prior to the microbial growth test study for specific samples, the procedure was tested and established using other soil samples. The mixture used in the procedure was that suggested by Alsop et.al (1980). The main considerations in establishing the procedure were the extraction process, the shaking period, the filter material and the bottles used in the experiment.

The soil extract was obtained from mixtures of 1:10; 1:5; and 1:2 (w/v) of soil and water. Besides measuring the turbidity, the heterotrophic plate count (HPC) of bacteria using R2A agar after dilution of soil extracts with Ringers solution was also conducted. The plates were incubated at 22°C for 7 days. Only 1:10 and 1:5 soil extract dilutions were examined by HPC, and involved 3 soil samples. The HPC results showed that the 1:10 dilution gave the higher number as did the turbidity results. Therefore the 1:10 (w/v) dilution was used through the experiment.

The second factor considered in the soil extraction process was the shaking period. Eight, 16, and 24 hours shaking periods at 180-200rpm in an orbital shaker were

compared to determine the maximum microbial extraction. Two soil samples were used in the test. Turbidity results showed that the 8 and 16 hours shaking periods performed with no significant difference whereas 24 hours shaking was significantly higher in soil sample 1 and lower in soil sample 2. With regards to making the experiment practical, a 16hour shaking period was selected for further experiments.

Another consideration was selection of the bottle to be used. Four types of bottle were compared, namely 100ml Erlenmeyer, 100ml medical flat bottle, 125ml small flat round bottle, and 250ml flat round bottle. Two soil samples were used in the test. Turbidity analysis demonstrated that the 100ml flat medical bottle always gave higher absorbance in both samples and therefore, it was decided to use this type for the microbial growth experiments.

To obtain the soil extract the soil slurry mixture was filtered before being applied in the experiments. The filtration of soil was carried out with GFA and Whatmann No. 1 filter paper. Comparing the turbidity results from these two filter papers showed that there was no significant difference between the filter papers. For economic reasons, the Whatmann No. 1 filter was used in the experiment.

Other tests related to the process of turbidity measurement after the mixing period were considered important. The effect of pouring or pipeting and shaking or not shaking the mixture prior to absorbance measurements was compared. Statistical analysis showed that directly pouring and not shaking the bottle indicated a higher absorbance. The procedure established was then as follows:

- i). soil extraction using 1:10 (w/v) ratio of soil:water mixture with 16 hours shaking and filtered with a Whatmann No. 1 filter paper;
- ii). microbial growth tests using 100ml flat medical bottle, 16 hours shaking period, the mixture unshaken and poured into the cavette prior to absorbance measurement by spectrophotometer.

8.3 Results of Experiments

8.3.1 Effect of nutrient types

Figure 8.1 illustrates the microbial population results after nutrient addition. N1 and N3 gave significantly higher counts than those shown by N2 and N4. The level of nutrient added also affected the microbial number. Only for N3 addition did the increase nutrient concentration also increase the population.

Comparing total bacterial count results of Soils 4 and 5, it can be seen from Figure 8.1 that Soil 5, in most cases contained more bacteria than Soil 4. Nutrients were added at concentrations similar to those in the respirometer soil slurry tests (see Pull-out Explanatory Note after page 176).

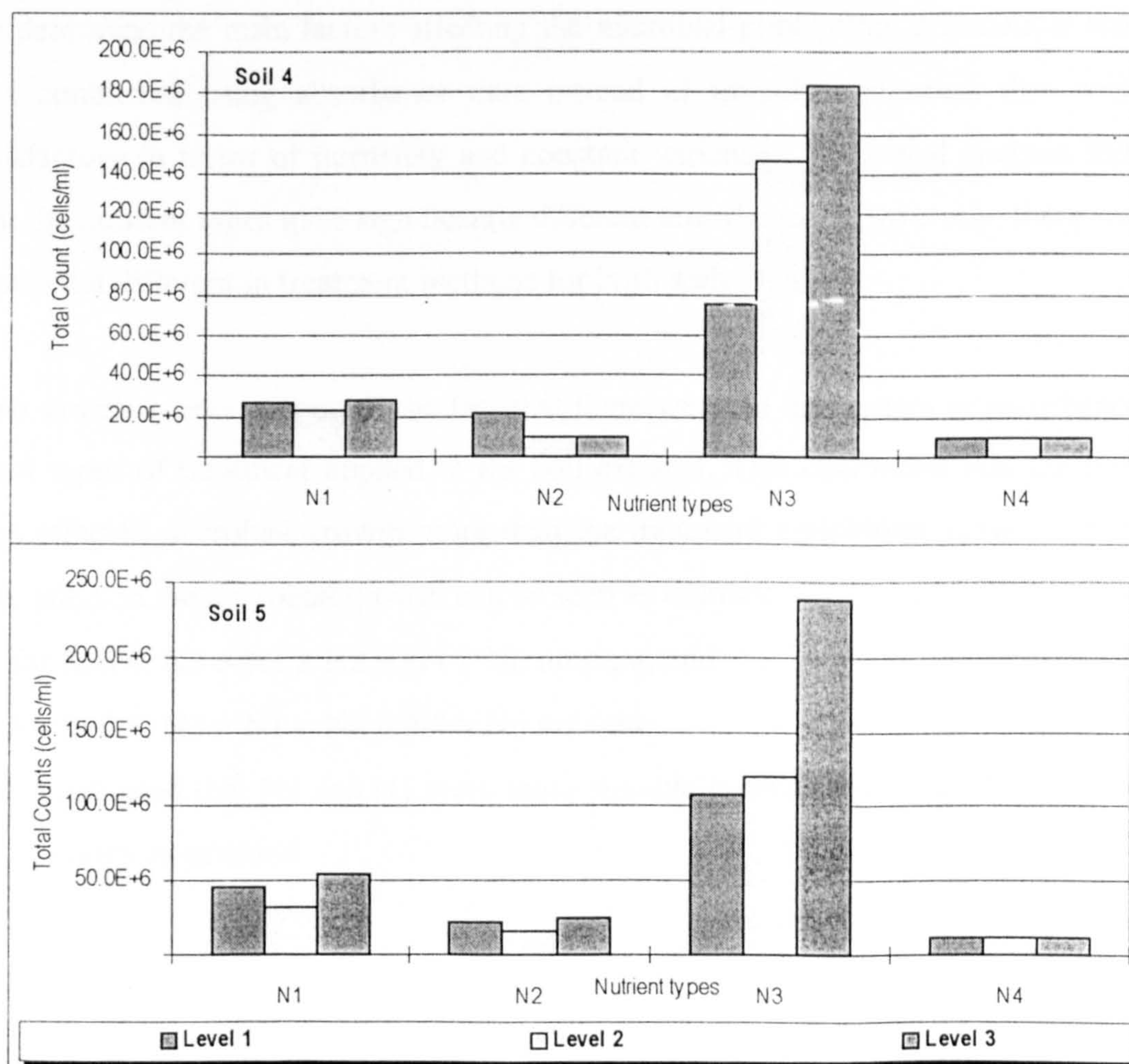


Figure 8.1 Microbial population after nutrient addition
(N=nutrients; Level 1= low; Level 2=medium; and level 3=high)

8.3.2 Effect of treatment method

The effect of the treatment method was investigated by application of nutrient only (Nut. only); nutrient + glucose (Nut+G); nutrient + glucose + acidified samples (Nut+G+A); and the condition if only acidification was used (A only). The nutrient was added at a medium level of concentration. The results shown in Figure 8.2 indicate that N3 addition resulted in the highest microbial population. For Soil 4 it can be seen that Nutrient 3 and glucose addition resulted in the highest growth whereas in Soil 5 this was found after treatment with Nutrient 3, glucose addition and acidification. Treatment without any addition was shown to result in lower microbial growth than that with added material. This suggested that the addition of nutrient and/or glucose significantly increased microbial growth in soil extracts.

To determine the main factors affecting the microbial population, a statistical analysis was conducted using absorbance data instead of microbial numbers that was not satisfactory in terms of normality and constant variance. Statistical analysis showed that the nutrient types gave significantly different absorbance. Conversely, there was no significant difference in treatment methods for both soils.

Two sample t-tests supported the fact that there were no differences in absorbance for the 4 types of treatment applied to the soil extracts. This concluded that the nutrient type affected microbial growth more than the treatment conditions. How the nutrient type affected the microbial growth can be seen as follows:

- i). for Soil 4: $N3 > N1 \geq N2 \cong N4 \cong \text{No nutrient}$, and
- ii). for Soil 5: $N3 > N1 \cong N2 \cong N4 > \text{No nutrient}$.

These indicated that N3 and N1 were more suitable for microbial growth than N2, N4 and no nutrient addition.

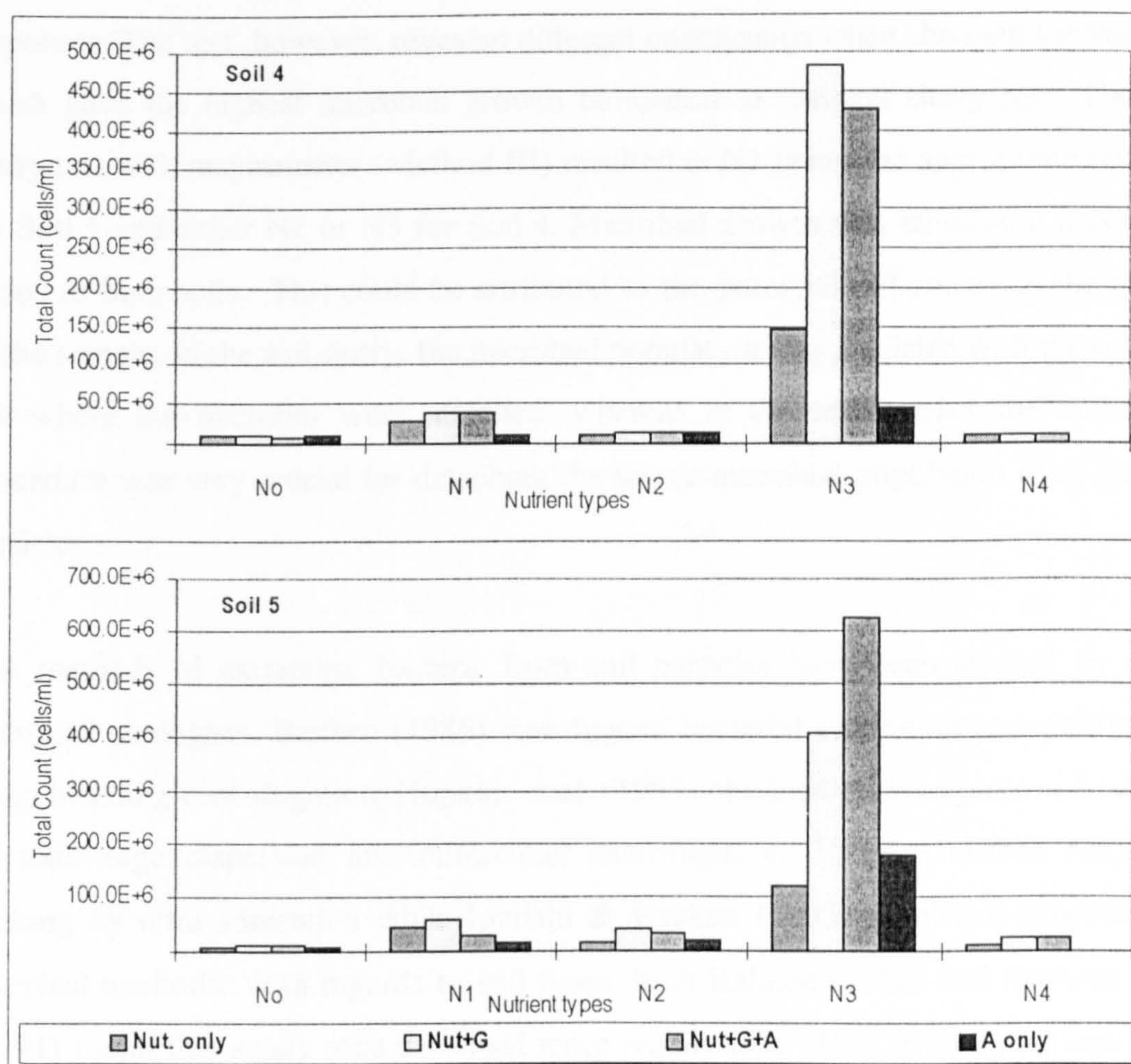


Figure 8.2 Microbial population in each treatment

(Nut.only=nutrient only; Nut+G=Nutrient+glucose, Nut+G+A=Nutrient+glucose+acidification; A only=Acidification only)

8.3.3 Effects of acidification

As shown in Figure 8.2 microbial growth in the acidified soil extract was no greater after nutrient addition. Only if acidification was accompanied by glucose and nutrient addition did microbial growth increase significantly, although growth was no higher than with unacidified+glucose+nutrient addition. This implies that acidification only of the soil extract did not result in the highest microbial growth.

8.4 Summary and Discussion

The microbial growth test was intended to investigate the appropriateness of nutrient and treatment conditions for the soil studied to differentiate between microbial growth

responses. The test, however, revealed different conclusions when showing the nutrient which gave the highest microbial growth compared to the soil slurry test. The soil slurry test with respirometer (Method III) resulted in N1 being the appropriate nutrient for Soil 5 and either N1 or N3 for Soil 4. Microbial growth was stimulated if N3 was added to both soils. This could be attributed to the potential differences in the nature of the sample. In the soil slurry, the microbial population was available with the original soil where the microbes were attached, whereas in the soil extract the extraction procedure was very crucial for detaching the whole microbial population from the soil particles.

The methods of extracting bacteria from soil particles have been studied by many microbial ecologists. Bakken (1985) investigated bacterial separation and purification using blending-centrifugation, Hopkins et al. (1991) obtained microorganisms from soil by multistage dispersion and differential centrifugation, Ramsay (1984) employed shaking by ultra sonication while Lindahl & Bakken (1995) combined physical and chemical methods. With regards to soil types, both Bakken (1985) and Hopkins et al. (1991) found that sandy soils dispersed more readily than clays. Bakken separated the microorganisms based on buoyant density and claimed that bacteria separated because of its high buoyant density. This was mainly due to the attachment of bacteria onto humic materials. Bakken further observed that the cells separated were negatively correlated with the clay content of the soil. Hopkins et al. (1991), on the other hand, found that they could recover 50% of the microorganisms from mineral soils (clay loam and sandy loam) which was better than from peat or organic soil.

This current study involved soil samples that are mostly classified as silty soils. Soil 4 was more sandy silt and Soil 3 was silty clay (Soil Laboratory – Dept. of Civil Engineering, Univ. of Newcastle upon Tyne). The separation method involved shaking the soil mixture in the orbital shaker for 16 hours at 1:10; 1:5 and 1:2 (w/v) dilution in distilled water. Bacterial counts using acridine orange at 0.01% (Hobbie, et al., 1977) resulted in soil extracts which contained 3.4×10^7 , 2.61×10^7 and 1.4×10^7 cells/g soil. Repeated measurement at 1:10 dilution for silty soil showed a bacterial count of 6.07×10^7 with a 2.5×10^7 standard deviation. Cell count results of the methods used by Hopkins et al. (1991) that involved multistage separation using a shaker, surfactants,

glass beads, blender, sodium cholate and ultrasonic yielded a bacterial population of around 6.5×10^8 , 1.8×10^8 , and 10×10^8 cells/g soil from clay loam, sandy loam, and peat soil respectively. These results showed an evidence that bacterial separation could be enhanced by the appropriate method being used.

However, methods of bacterial separation usually consider options for releasing the microbial population and at the same time maintaining the survival of cells for further tests or experiments. Lindahl and Bakken (1995) noted that bacteria in dormant or non-growing state were more resistant to an external stress factor such as sonication. The use of 'rotating plaster' treatment and a Waring Blender were considered to do no harm to the majority of soil bacteria even after long periods of treatment. The choice of separation methods thus relied on the purpose of cell extraction. If the soil extract was for metabolic status, growth potential, or viability, Lindahl and Bakken suggested the use of straightforward mechanical dispersion such as a Waring Blender in distilled water. However, it should also be borne in mind that some bacteria such as *E.coli* have been shown to be destroyed by 18min shaking in Waring Blender.

The extraction procedure in this study considered the shaking period and the intensity of shaking. During the establishment of the appropriate test procedure, it was noticed that the first orbital shaker used had limited rotation and the performance of the shaker decreased after a long period of shaking. With a slower rotation, the soil extract obtained resulted in a very low microbial detachment. The turbidity results were much lower than those with the best shaker operation. Therefore, the orbital shaker was replaced by a new system which was more accurate and had higher capabilities. The microbial growth study of Soils 4 and 5 was carried out using a digital orbital shaker.

Growth in soil extract was also attempted using heterotrophic plate counts with R2A agar at 20°C for 7 days. For Soils 1, 2 and 3, a dilution of 1:10 after 8hrs shaking resulted in cell counts of 2.3×10^4 CFU (6.3×10^3 STD). This gave around 0.7% of the total bacterial counts. In Soils 4 and 5, the percentage of the platable microbial count was found to be at an average of 6% of the total counts using acridine orange (or in the range of 0.9 to 14%). This was in agreement with Aelion and Long (1994) who obtained 1-10% of the total cell counts and Hopkins et al. (1991) who were able to

obtain 2% platable microorganisms in homogenated samples (i.e. the first step of a multistage separation method they proposed) and 2.5 to 5% in supernatants after repeated blending and centrifugation.

Comparing Soils 1 and 2, which were silty and had a relatively similar structure and Soil 3, which was more clay-like showed that the plate count result of Soil 3 was slightly higher. Although Soil 3 was clay-like, the higher result was generally related to the origin of the soil sample. Soil 3 was collected as topsoil and classified as uncontaminated. Moreover, it contained roots and other portions of small plants.

The study used turbidity and absorbance as the estimate of microbial population in the investigation of appropriate nutrients for microbial growth. Other common methods to determine the appropriate nutrient for microbial growth which have been used in a feasibility study of bioremediation involved the measurement of cell weight or bacterial counts. Anderson (1995) referred to the work of Fiorenza (1991) for the bioremediation of subsurface material using liquid delivery by cell weight comparisons. Leavitt in Anderson (1995) used microbial density as the CFU of heterotrophs and hydrocarbon-degrading bacteria in a biotreatability study of diesel fuel bioremediation. Watwood and Carr (1994) also applied the CFU of heterotrophs in their study to investigate the most appropriate nutrients.

The current study tried to carry out a further investigation on the types of microbial population by Gram staining of the mixtures. The stains showed that a different bacterial population was found in mixtures with different nutrient type additions. Some colonies which were detected in those mixture can be seen in Plates 8.1 - 8.3. The microbial staining pictures were taken using an Olympus camera with ISO 100 film, at 5seconds exposure. Similar exposure time was also used by Ray (1995) for the same microscope and camera. The plates showed that different nutrients gave different types of colony grown in the medium.

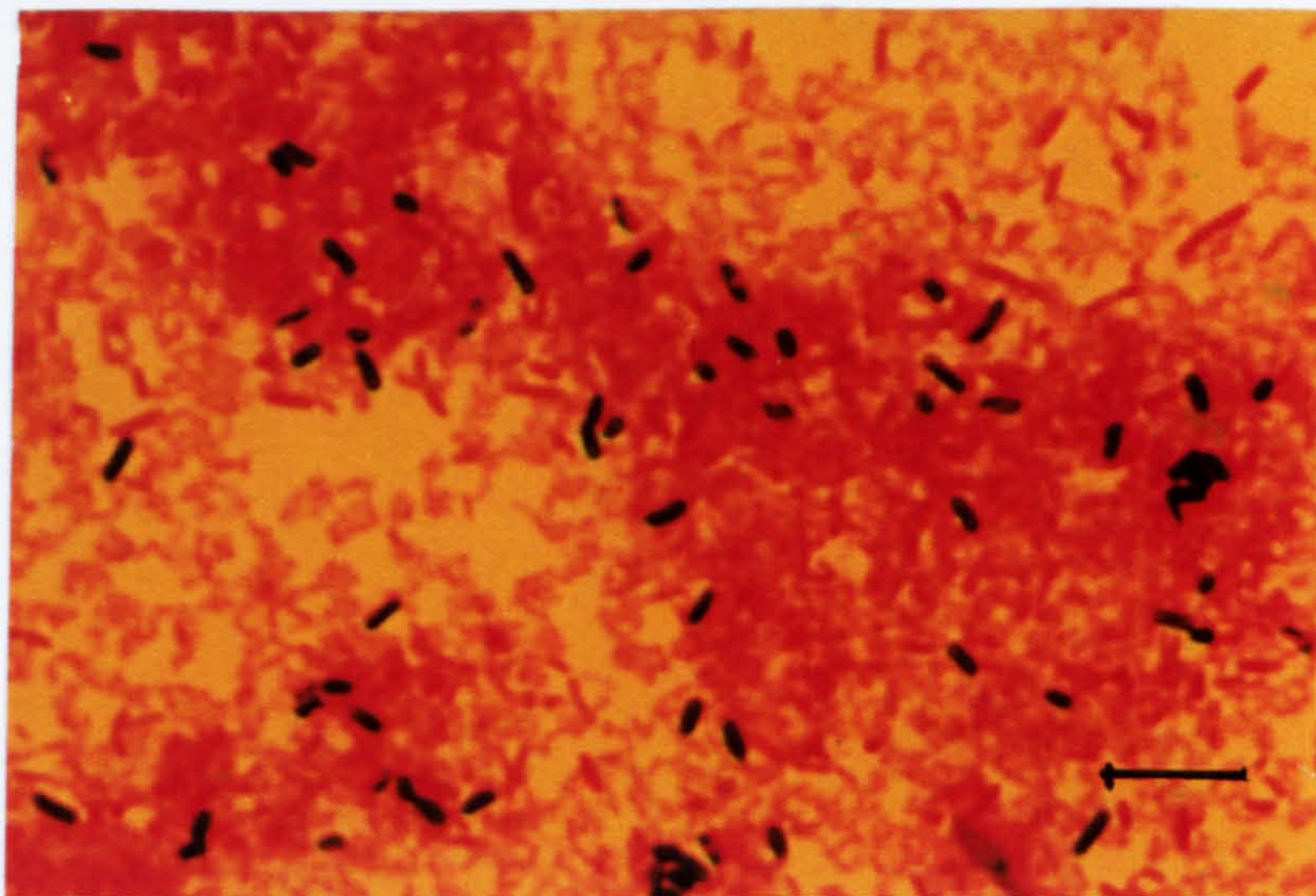


Plate 8.1 Bacterial morphology of mixture with N1 addition (Bar=100 μ m)

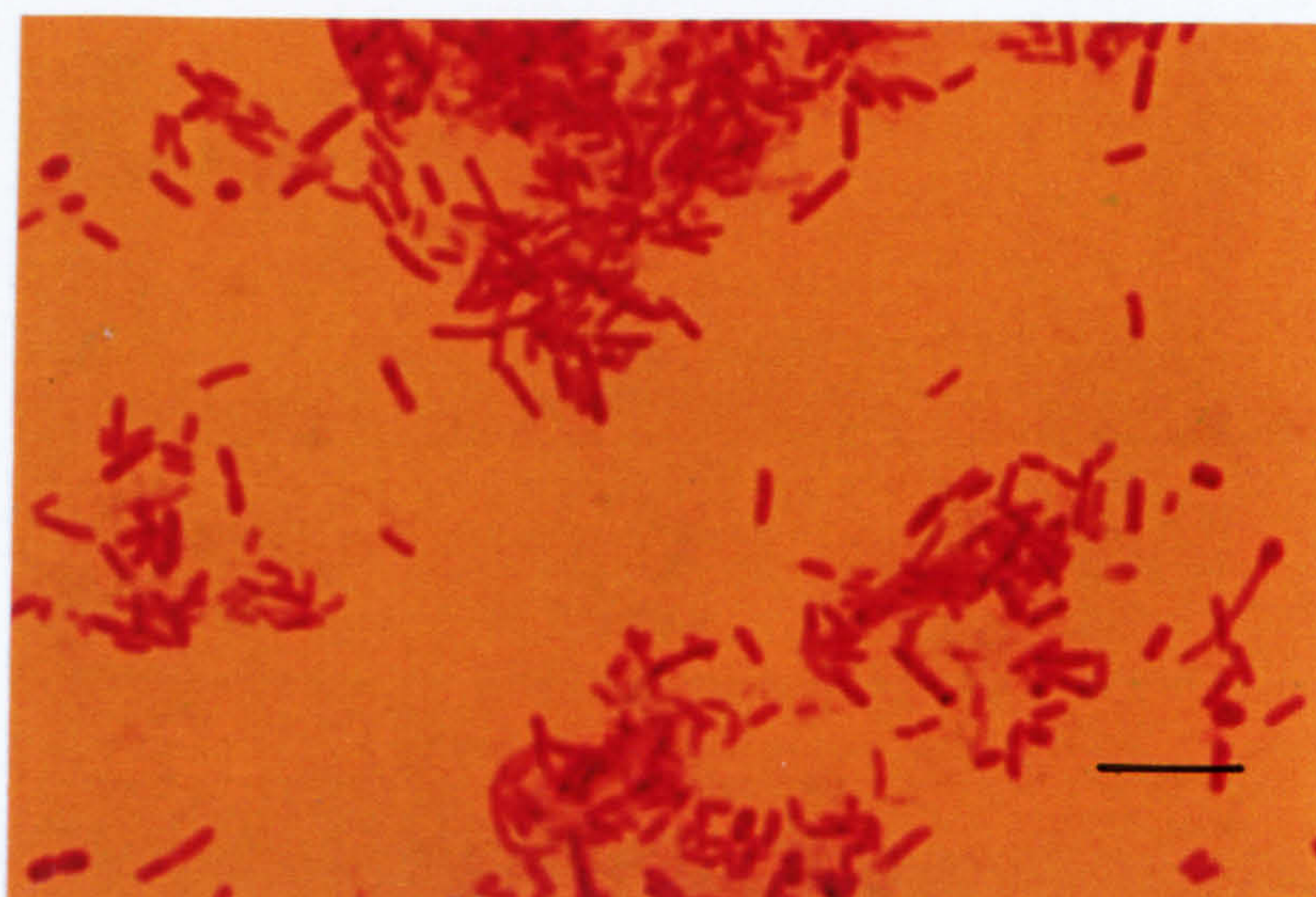


Plate 8.2 Bacterial morphology of mixture with N2 addition (Bar=100 μ m)

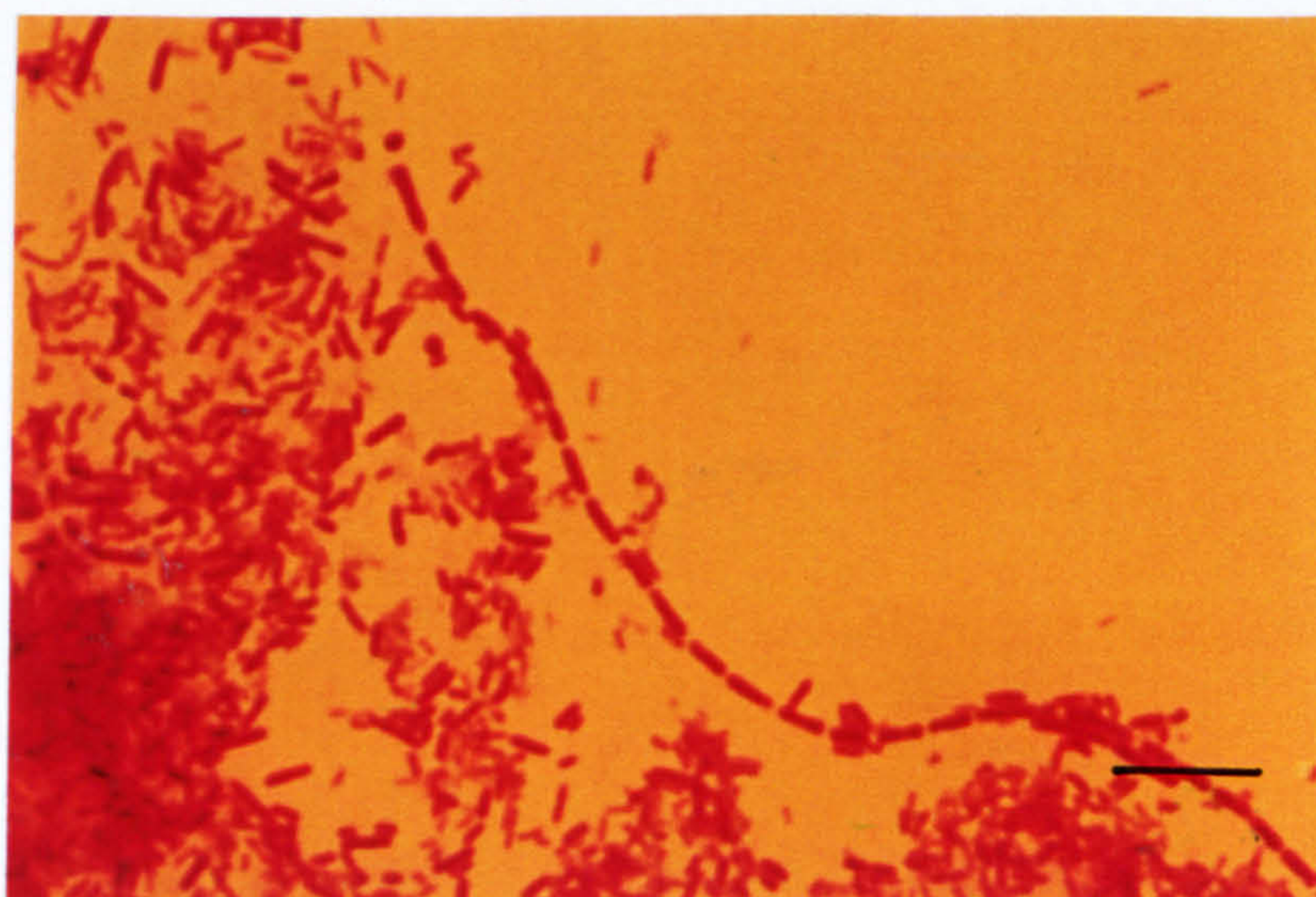


Plate 8.3 Bacterial morphology of mixture with N3 addition (Bar=100 μ m)

8.5 Conclusions

In the microbial growth test, measuring absorbance as the assessment of turbidity in soil extract mixtures showed that for both soils samples N1 and N3 resulted in a higher microbial population than other nutrients. The addition of N3 stimulated growth significantly better than N1. The N4 supply contributed the smallest effect and was similar to the results without any nutrient addition. For N3 addition, the greater the amount of nutrient added, the higher was the microbial count.

The addition of glucose similarly increased the bacterial numbers. It could be concluded that glucose was likely to be the rate-limiting factor as it boosted the microbial numbers and also its respiration activity. Results of the microbial growth tests after acidification did not increase the microbial population unless glucose and nutrient was also added. The increase in microbial population was even greater with this combination in Soil 5 (see Fig. 8.2). This could be related to the optimum environment for Soil 5 to enhance the growth of sulphate reducing bacteria since sulphate was found to be lower in acidified soil +glucose addition.

Both Nutrients 1 and 3 demonstrated significant response compared to other nutrients. To some extent this was also shown in slurry tests. Despite higher microbial growth being found for N3 (P only source) addition, N1 (with N and P sources) was selected for column test. N1 produced higher effects in both slurry and also microbial growth. In addition, glucose at the lowest concentration was also being added in the soil columns.

CHAPTER 9

RESULTS AND DISCUSSION OF ANAEROBIC BIOREMEDIATION IN SOIL COLUMN

9.1 Background

The main objective of the column tests was to investigate the response of the addition of material into the soil studied in the solid phase. Rogers et al. (1993) stated that reaction in the slurry phase is a maximum due to its higher contact surface compared to the solid phase. Moreover, the soil column experiment was considered to be closer to the situation at the Gateshead site. This, however, can only be accepted if the sample used was an undisturbed soil column. Therefore, in this study the conclusions will only be applied to specific homogenised soil samples and not those in the original site.

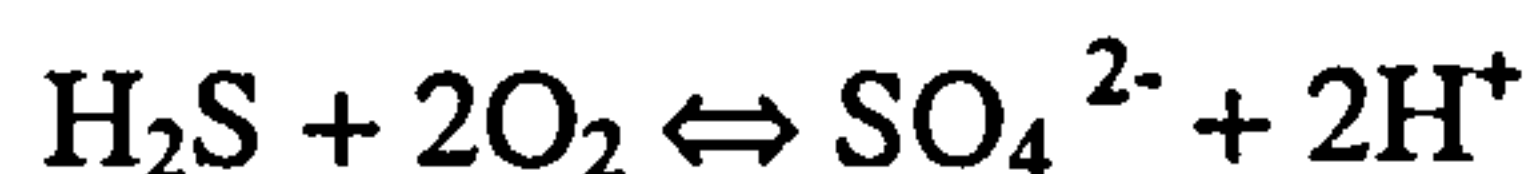
The soil column was tested using 4 types of treatment, namely: deionised water addition (Treatment I); deionised water and air (Treatment II); Nutrient 1 (contained N and P sources) addition (Treatment III); and glucose + Nutrient 1 addition (Treatment IV). These types of treatment were carried out sequentially. The soil slurry and microbial tests concluded the appropriate environment for sulphate reduction was an anaerobic condition, N and P nutrient (Nutrient 1) and glucose addition. However, the column was initially designed to accommodate aeration process. Despite the results from the slurry test, treatment with the aeration process (Treatment II) would still be included as a confirmation of the slurry test.

Nutrient 1 was selected from the results of the gas and CO₂ evolution in the soil slurry and also microbial growth tests. The nutrients in the experiments were a mixture of concentration of NH₄HCO₃ (20mg/l), KH₂PO₄ (10mg/l) and 12mg/l of K₂HPO₄. This was double the strength of similar nutrients used by Irvine et al. (1993) in their soil slurry reactor study. The experiments analysed soil quality and leachate generated from both columns.

9.2 Results of Experiments on Leachate Collected

9.2.1 pH response to treatment

A comparison of the pH response in the leachate for each treatment is illustrated in Figure 9.1. Soil Column 1 performed slightly differently from Column 2. The pH from Column 1 appeared to be affected by the treatments. The changes were quite obvious because its initial pH was very alkaline. Treatments I and II did not clearly change the pH whereas in Treatment III (nutrient addition) and Treatment IV (glucose+nutrient), the pH reduced considerably. Treatment III changed the pH from around 12 to 9.4 while in Treatment IV the pH decreased from 9.8 to 7.62. This suggested that there were some changes in metabolism in the soil system. According to Howarth et.al. (1992) most oxidation of sulphur produces protons or acidity. During sulphate reduction, reduced sulphur is stored in the sediment. If the reduced sulphur is re-oxidised to sulphate, acidity is produced. The chemical reaction of re-oxidation of reduced sulphur is:



On the contrary, in Soil Column 2, the pH changes were not as dramatic as in Column 1. In Treatment I the pH only changed from 7.6 to 8.9 whereas in Treatments II, III, and IV, the pH of the leachate was almost constant (around 8.3; 8.3 to 8.0; and around 7.9 respectively).

9.2.2 Sulphate concentration response to treatment

Changes of sulphate concentration in the leachate of the soil columns are illustrated in Figure 9.2. As for the pH results, the sulphate concentration changed markedly in Column 1. Although, this is not so graphically clear there was a difference in sulphate concentration from Treatments I and II. The sulphate concentration in the leachate reduced from 970 mg/l to around 53 mg/l at the end of the Treatment I experiment (leaching with deionised water). On the other hand, Treatment II (deionised water and aeration) changed the sulphate concentration from 50 mg/l to 109 mg/l. An interesting result was seen after the addition of nutrient whereby the sulphate concentration increased significantly from 704 mg/l to 10770 mg/l at the end of Treatment III. The

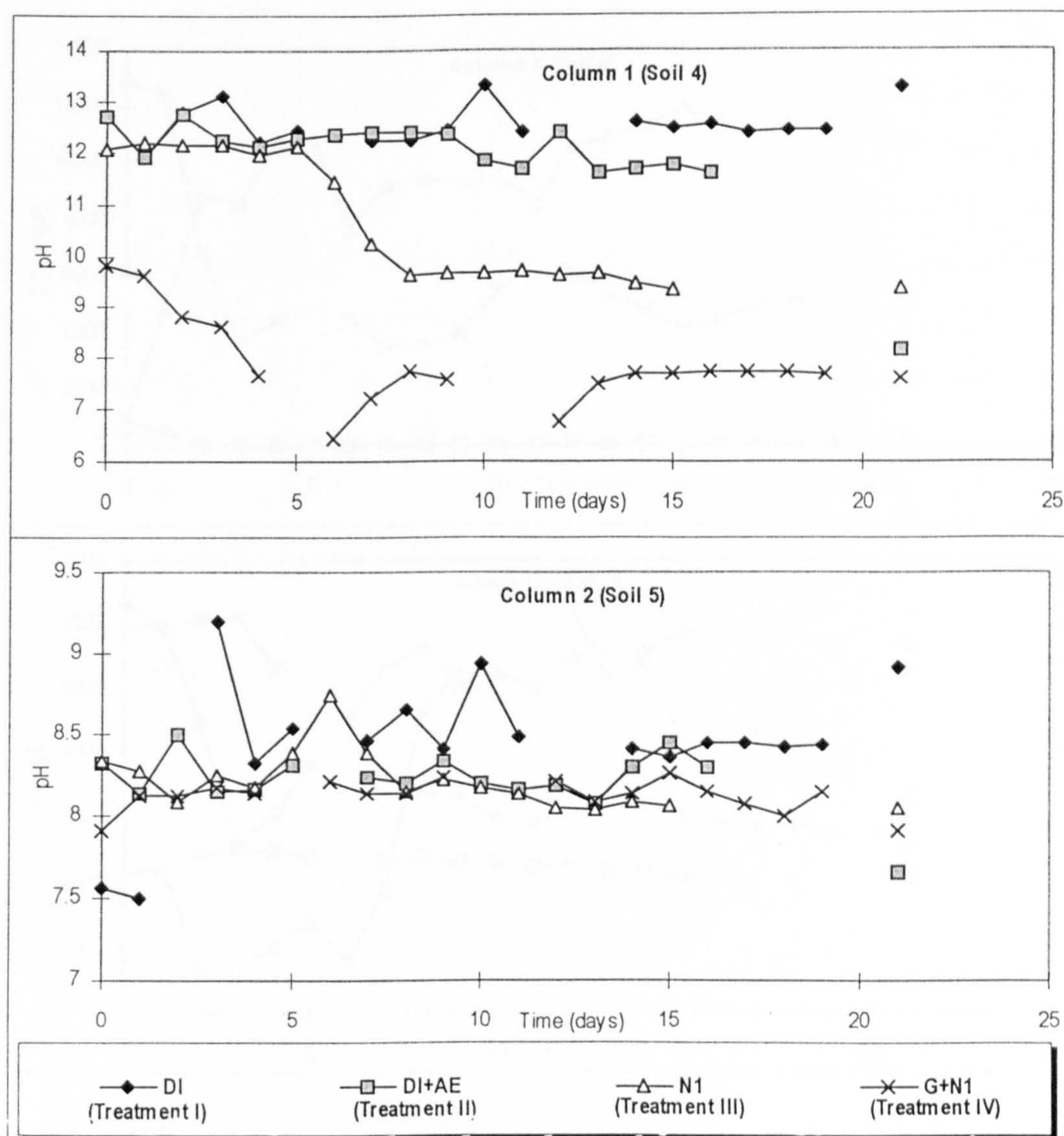


Figure 9.1 Response of pH in leachate after treatment in soil columns

nutrient addition did not encourage microbial action to reduce the sulphate. There was oxidation or mineralization instead. However, the addition of glucose and nutrient as Treatment IV showed a reduction of sulphate from 12870 mg/l to 5030 mg/l (around 61%).

In Column 2 similar results were observed. Deionised water appeared to wash the soil and significantly leached sulphate at the beginning. The reduction in sulphate concentration was around 60% (from 6300 mg/l to 2500 mg/l). In Treatment II, where deionised water and air were supplied, the sulphate concentration in the leachate was almost unchanged. The reduction was only about 20% (from 2500 mg/l to about 2000 mg/l). In contrast, nutrient addition (N1) to the soil column released more sulphate to the leachate. It was found that the leachate contained about 2500 mg/l sulphate at the

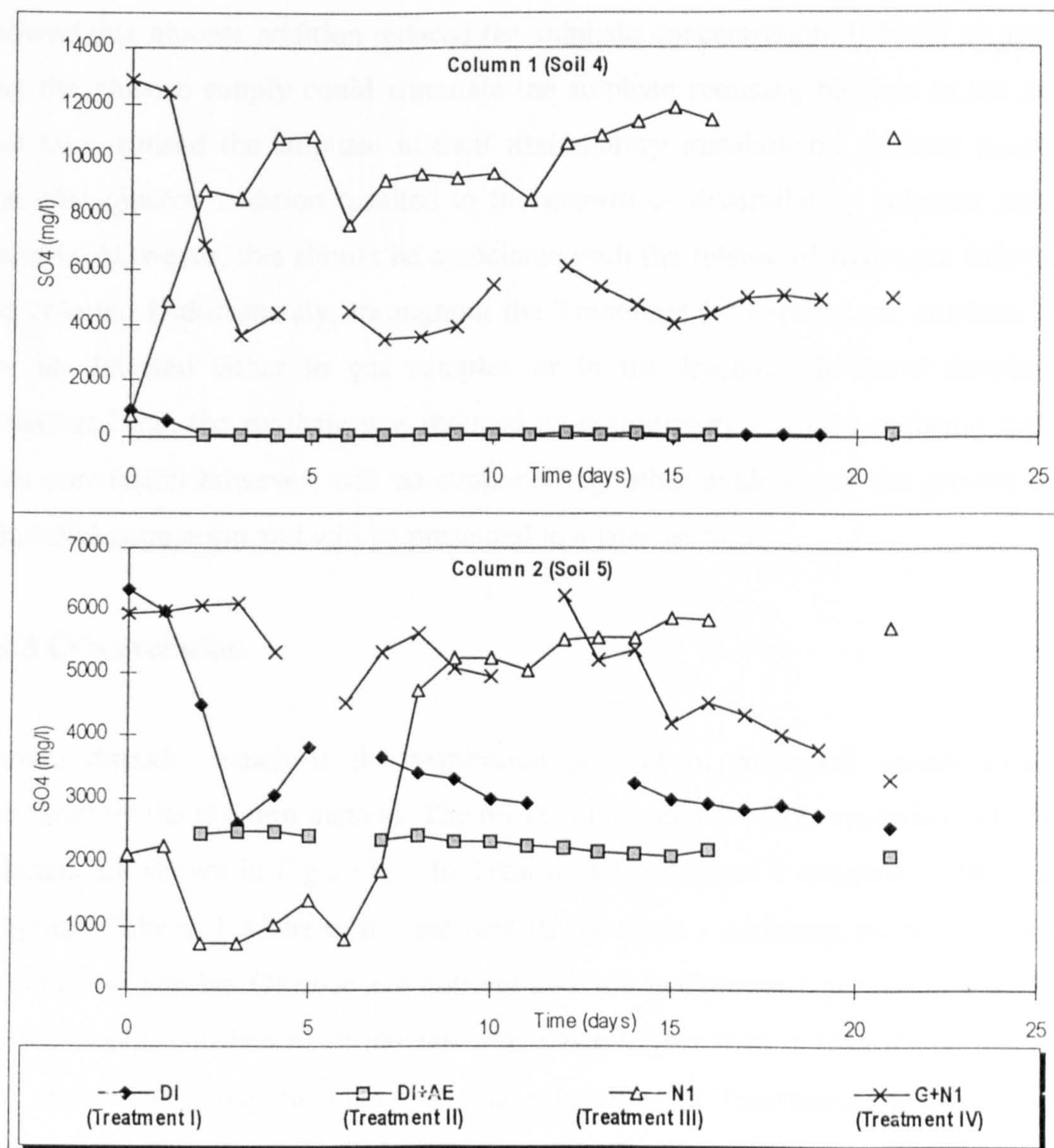


Figure 9.2 Response of sulphate in leachate after treatment in soil column

beginning and after 10 days of nutrient supplied, the sulphate available doubled to about 5000 mg/l. Although some reductions of up to 60% were also observed after 7 days, sulphate was later generated at a higher concentration. This concentration increased slowly until it reached about 5700 mg/l at the end of the experiment. Treatment IV, in which glucose was added as another carbon source, showed that the sulphate concentration could be reduced again from 5900 mg/l to 3300 mg/l in the leachate. This amounted to around 44% reduction.

These sulphate levels in the leachate demonstrated that nutrient addition was not encouraging for sulphate immobilisation. On the contrary, nutrient addition considerably increased sulphate in the leachate. Furthermore, the column study also

showed that glucose addition reduced the sulphate concentration. It could be assumed that the glucose supply could stimulate the sulphate reducing bacteria in the soil so that they utilised the sulphate in their assimilatory metabolism. Another possibility was that glucose addition resulted in the growth of dissimilatory sulphate reducing bacteria. However, this should be associated with the release of hydrogen sulphide in the column. Unfortunately, throughout the Treatment IV experiment, sulphide could not be detected either in gas samples or in the leachate. It could therefore be considered that the sulphate was reduced by assimilatory sulphate reducing bacteria. This conclusion however, will be supported by other evidence of the growth of the microbial population and will be presented in a later section.

9.2.3 CO₂ evolution

Carbon dioxide, which is the respiration product of microbial metabolism, was measured by the titration method. The results of cumulative CO₂ evolution in both soil columns are shown in Figure 9.3. In Treatment II, Column 2 released 10 times more CO₂ than Column 1 whereas in Treatment III (Nutrient 1 addition), eventually the CO₂ evolved was similar. Glucose and nutrient addition in Column 1 generated less CO₂ at the beginning, but then its production was much higher than in Column 2. The graph also showed that the magnitude of CO₂ evolution in Treatment III was very low compared to Treatment IV. This indicated that the nutrient was not the rate-limiting factor. In addition, for Column 2, aeration and deionised water addition gave a higher cumulative CO₂ than nutrient addition alone. Both soils required a carbon source to enhance the microbial respiration (see Figure 9.4).

Although Column 1 originally had an almost similar microbial population (total bacterial count of 4.43×10^5 cells/g soil compared to soil Column 2 with 5.37×10^5 cells/g soil), the CO₂ production in Column 1 for Treatment IV was almost double that in Column 2.

Besides titration, CO₂ was also measured in gas samples before the CO₂ traps. The proportion of CO₂ was noticeable and detectable in Treatments III and IV. The

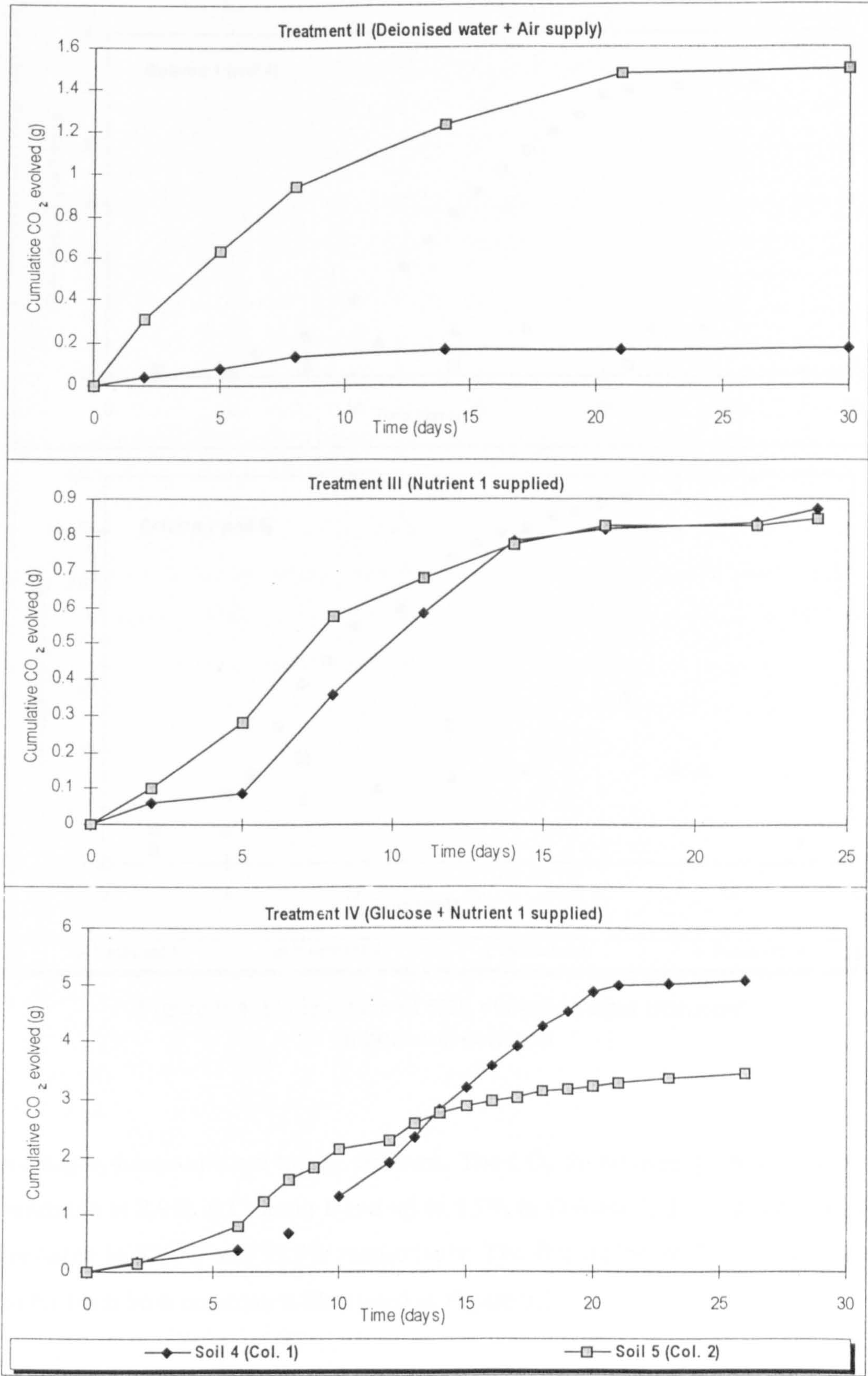


Figure 9.3 Comparison of CO₂ evolution in both soil columns after Treatments II, III and IV

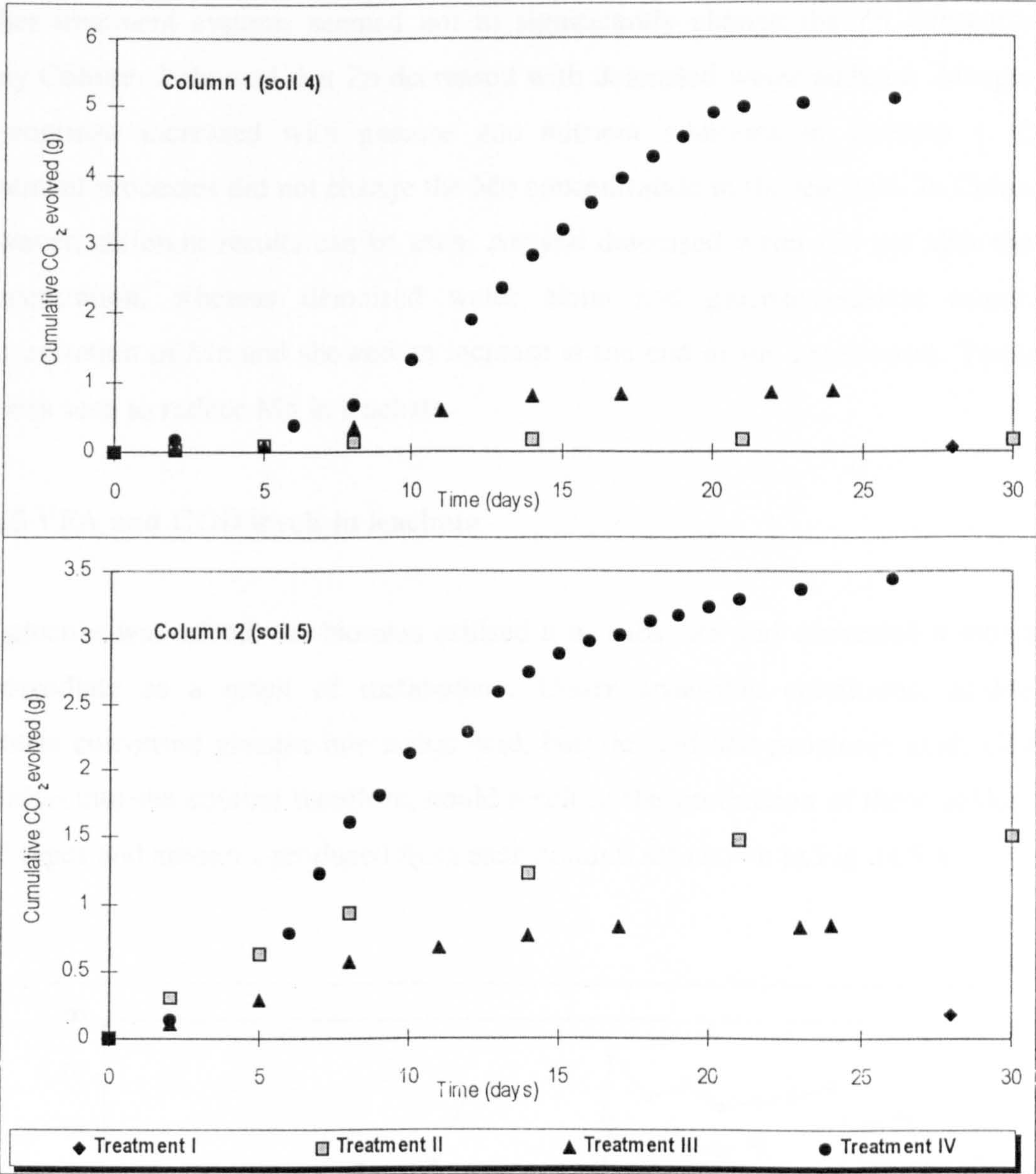


Figure 9.4 Comparison of CO₂ evolution after treatment in both soil columns

percentages, however were totally different. The CO₂ found during Treatment III was a maximum at 2.9% in Column 1 and up to 5.7% in Column 2. In contrast, Treatment IV resulted in 83.7% and 72.9% respectively. The fluctuation of CO₂ evolved in gas samples from both columns is illustrated in Figure 9.5.

9.2.4 Leaching of Zn and Mn

The zinc and manganese concentrations in the leachate are shown in Figures 9.6 and 9.7. Similar to sulphate, Zn was leached more after nutrient treatment in Column 1.

Other treatment systems seemed not to significantly change the Zn concentration. Only Column 2 showed that Zn decreased with deionised water addition. Manganese, in contrast, increased with glucose and nutrient additions in Column 1. Other treatment processes did not change the Mn concentration in the leachate. In Column 2, however, different results can be seen. Air and deionised water did not alter the Mn concentration, whereas deionised water alone and glucose+nutrient raised the concentration of Mn and showed an increase at the end of the experiment. Treatment III was seen to reduce Mn in leachate.

9.2.5 VFA and COD levels in leachate

As glucose was added, the biomass utilised it as substrate and converted it into some intermediate as a result of metabolism. Under anaerobic conditions, acidogenic bacteria converted glucose into acetic acid, butyric acid and propionic acid. Glucose addition into the column therefore, could result in the production of these acids. The acid types and amounts produced from each column are shown in Figure 9.8.

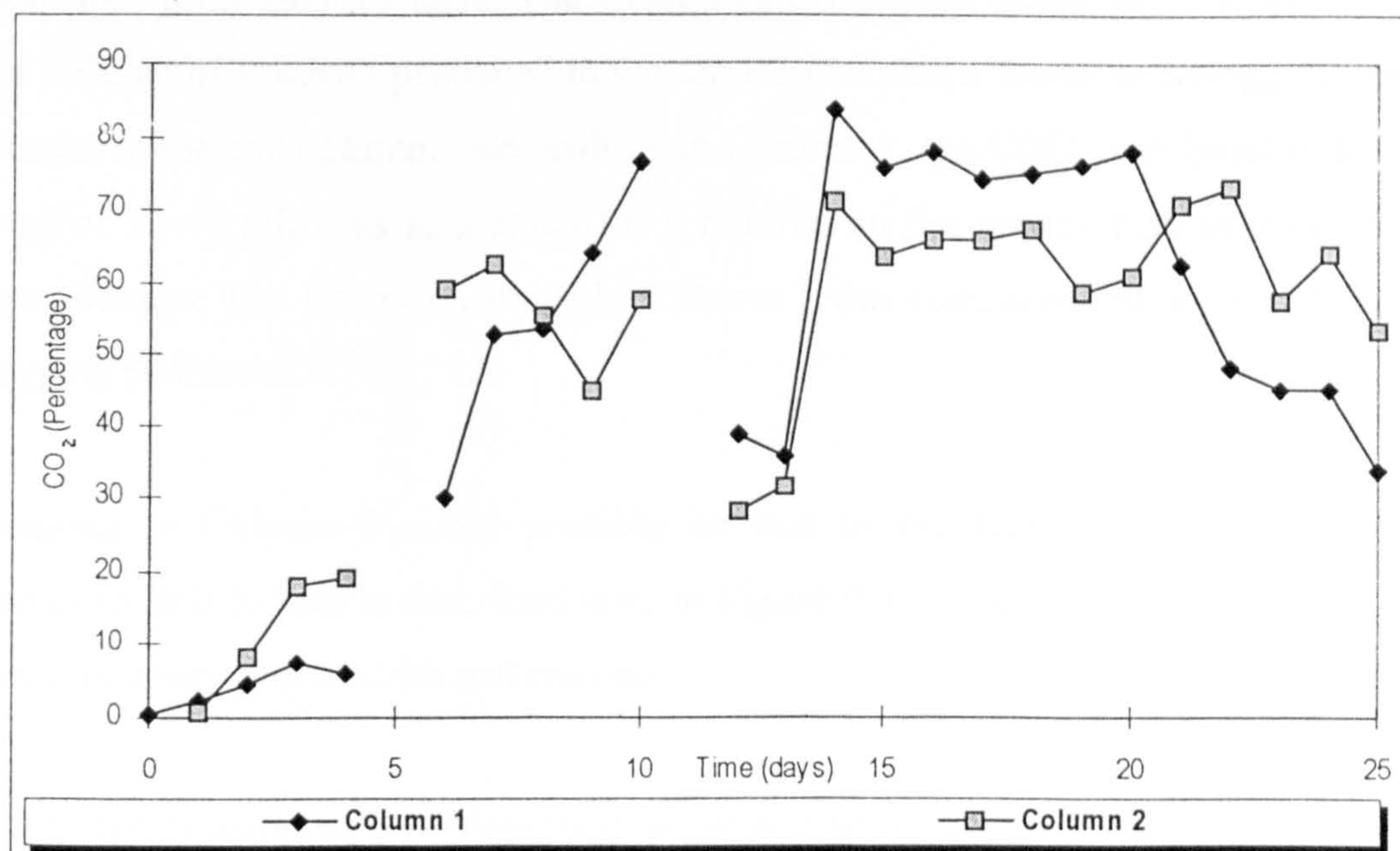


Figure 9.5 Cumulative CO₂ evolution in gas samples after Treatment IV

Column 1 showed greater acid concentration and types. Acetic acid was produced at first followed by iso-butyric acid, and n-butyric acid. Column 2 was dominated by acetic acid production.

Glucose was added at 2500µg C /g soil (Wu et al., 1995). The COD was also measured in order to observe the reduction in organic content. Due to the different density and permeability of the soil samples in each column, the volume of glucose added was not similar and consequently, the volume of leachate generated was also different. The weight of dried soil up to certain height was measured for each column. Similar height of soil column was obtained by addition of deionised water. This procedure noted differences in soil permeability. The glucose was then added in accordance with the total soil weight in the column.

The COD released in the leachate was also different since the glucose added differed. Column 2 received a higher glucose strength due to its lower permeability and higher soil density. The COD of the feed in Column 1 was 45 g/l whereas in Column 2 it was 180 g/l. The COD in the leachate from Column 2, however, was always lower than 1000 mg/l until after 17 days. The COD was eventually found to be around 3- 4g/l. The volume of leachate produced in Column 2 was much lower and clogging seemed to occur in the soil column. Meanwhile, in Column 1, the COD was found to be in the range of 17-47 g/l. This accounted for a removal in the range of 30 to 60% after 13 days addition. The leachate volume in Column 1 was considered to be less affected by clogging problems.

Clogging in Column 2 could possibly be due to the higher microbial population density in Soil 5. This is described later in Figure 9.12 which shows the total bacterial count for every port in each soil column.

During the experiment with deionised water and aeration (Treatment II), it was found that the leachate in Column 1 was covered with a layer which could be broken after interruption and settled later. The layer was then found to be a calcium precipitate which was formed after being exposed to the atmosphere. If the calcium was aerated with CO₂, the white flake of calcium carbonate precipitated (Holderness and Lambert, 1966). In addition, according to Manahan (1994), the calcium sulphate deposits could

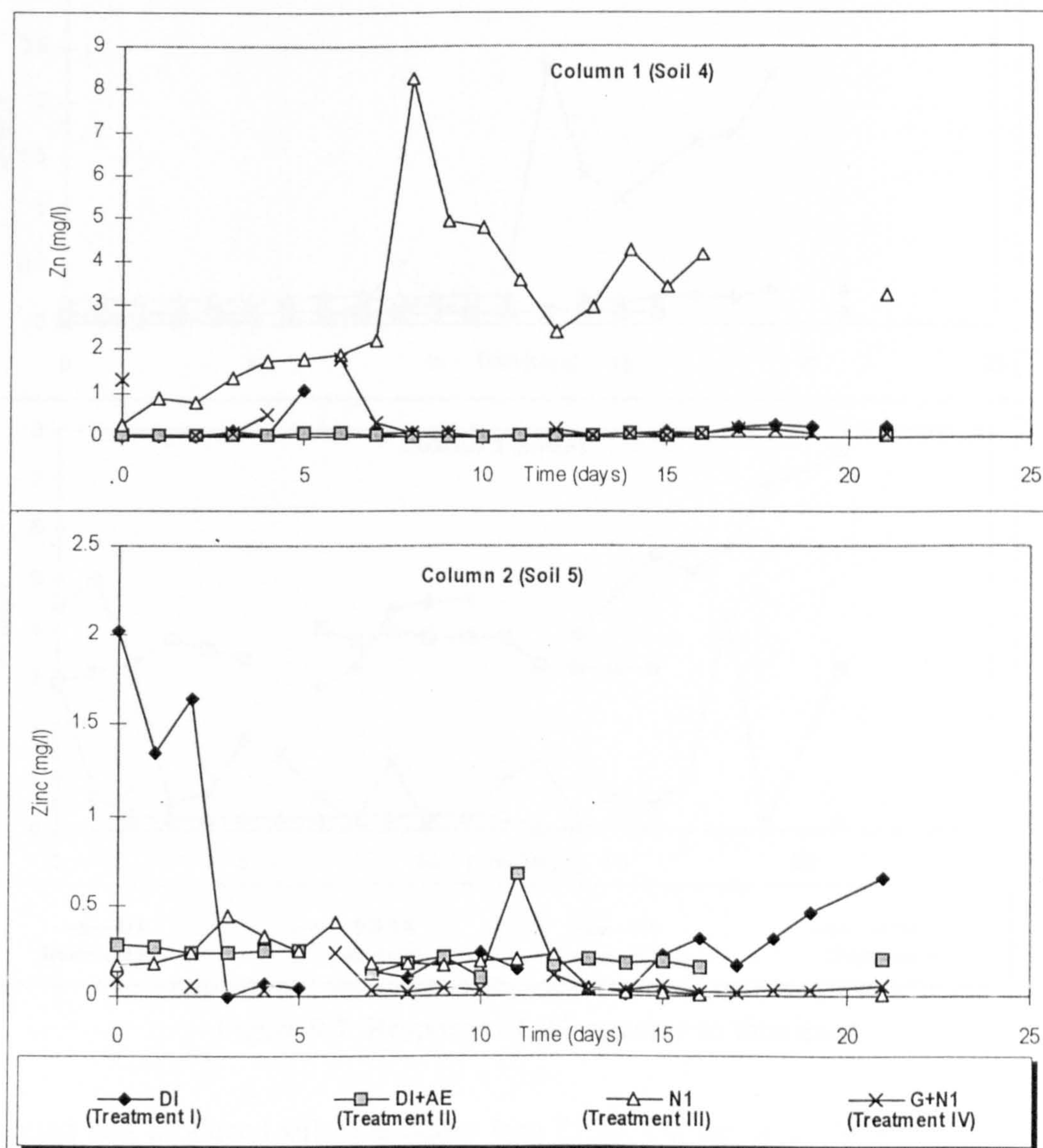
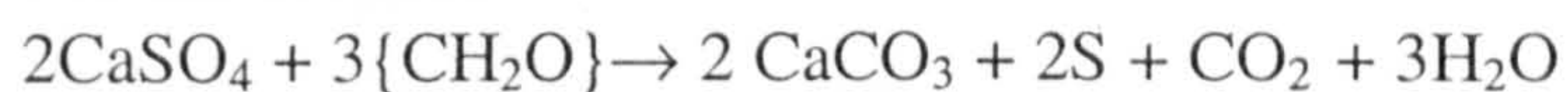


Figure 9.6 Response of Zinc to treatment

be bacterially reduced to form elemental sulphur and which was then interspersed in the pores of the limestone products.



Although the free sulphur in the deposits was never found to be due to the formation of escaped volatile H_2S .

The study, however, could not detect any sulphide gas during the experiment. The pH of soil samples were 8 and 11. In this pH the pressure of H_2S was too small to be

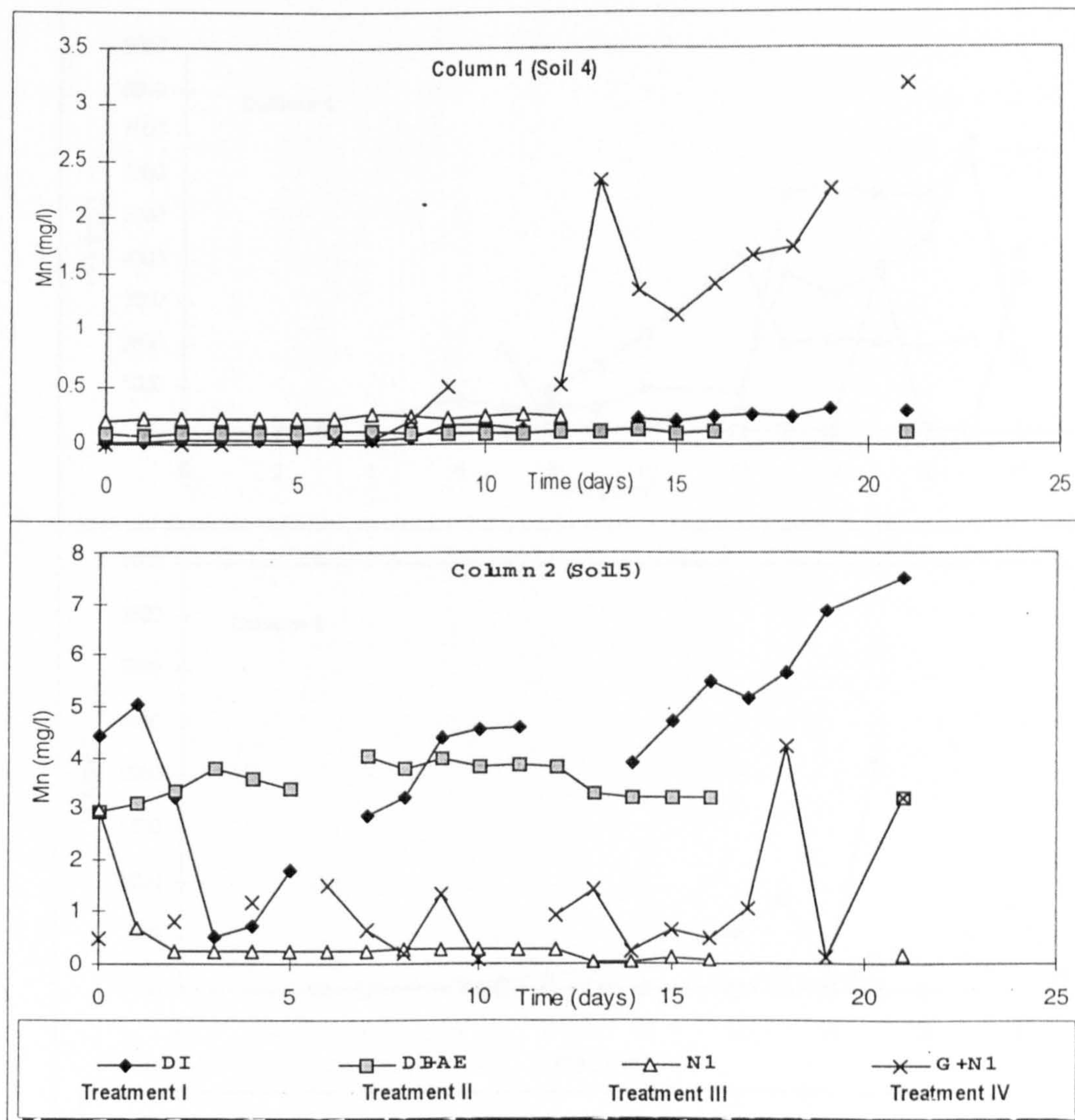
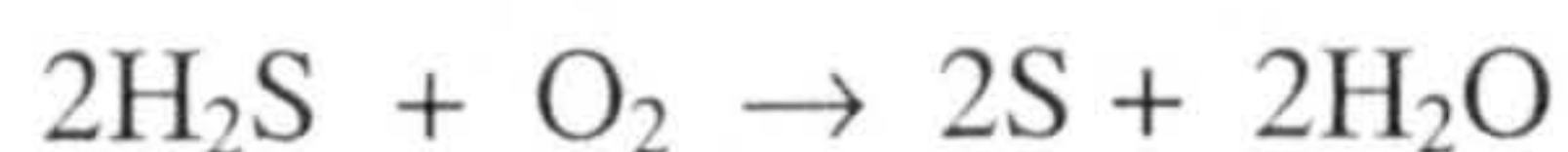
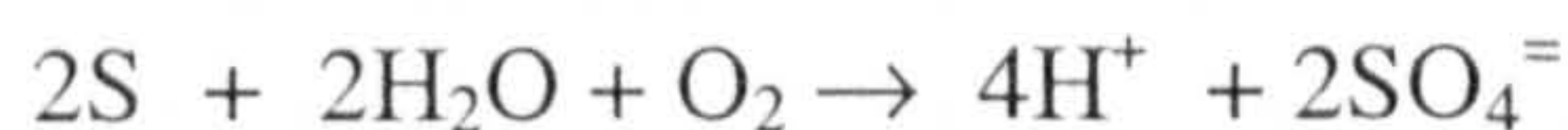


Figure 9.7 Response of Manganese to treatment

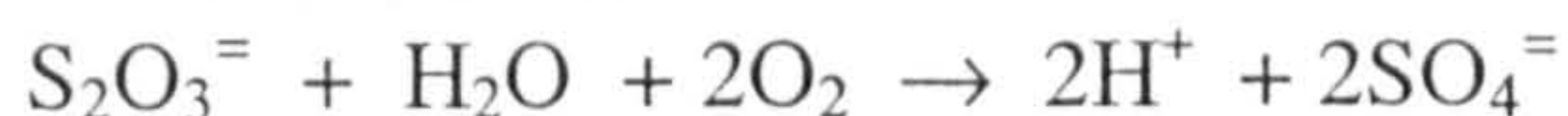
detected and produced sulphide odour (see §7.2). On the other hand, the H_2S could also be reoxidised by sulphur bacteria:



Elemental sulphur could also be oxidised to form sulphate under aerobic conditions:



or by oxidation of thiosulphate:



Therefore, with aeration (Treatment II) of Column 1 the pH was lower at the end of experiment. The sulphate concentration was doubled in Column 1 (see §9.2.2).

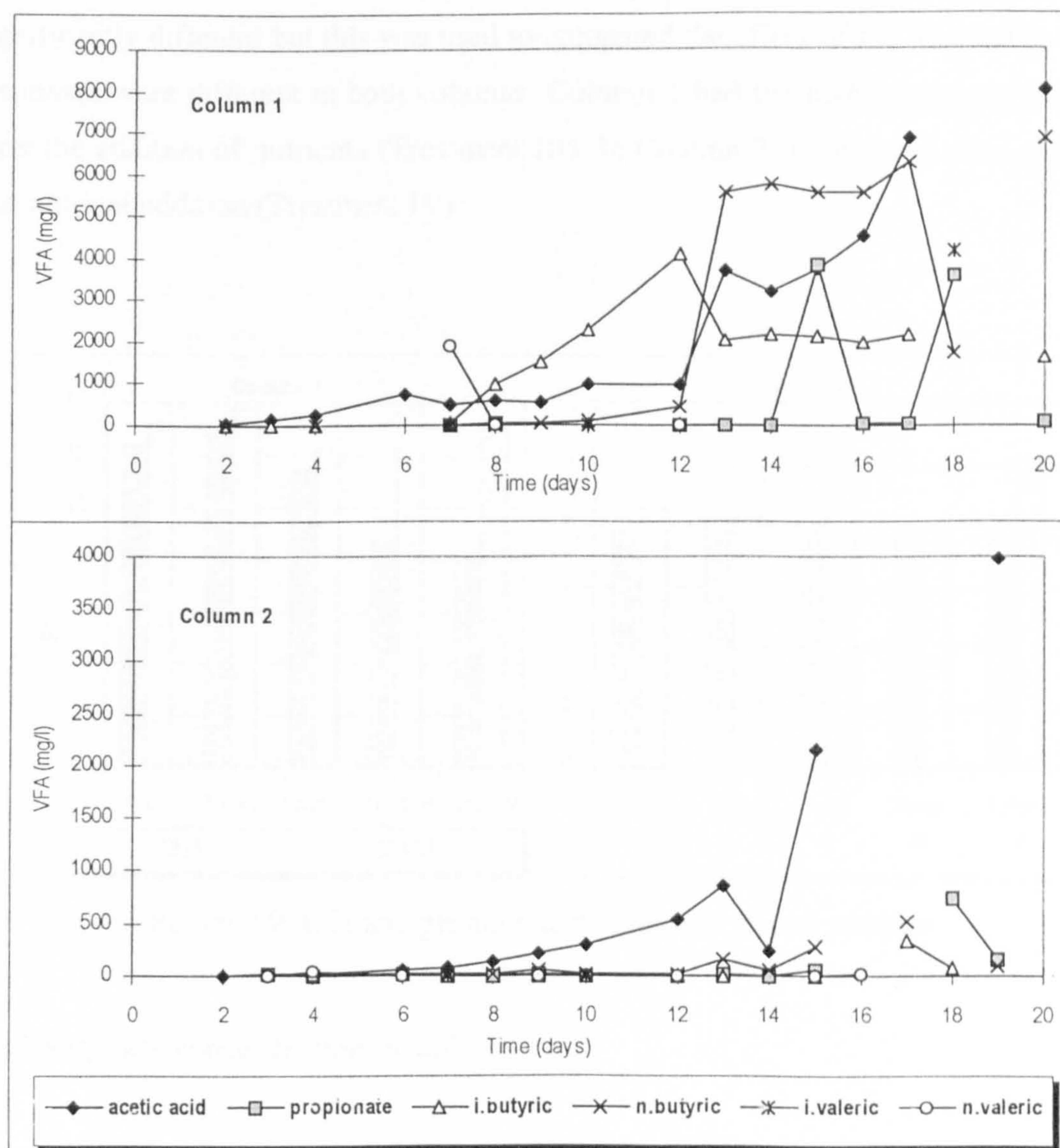


Figure 9.8 Volatile fatty acids in leachate after glucose addition in columns

9.3 Results of Experiments on Soil from the Columns

After each treatment the soils were sampled from the columns, dried and analysed. Chemical and microbiological quality such as pH, organic content, sulphate, heavy metals and microbial population were measured.

9.3.1 Organic content and pH in soil

Figure 9.9 shows a comparison of the pH value and organic content as LOI (loss on ignition) in each column. There was a decrease in pH in Column 1 after treatment whilst the pH changes in Column 2 were not as marked. Although the LOI was not

significantly different but this was used to compared the effect of the treatments. LOI responses were different in both columns. Column 1 had the highest organic content after the addition of nutrients (Treatment III). In Column 2, it appeared after glucose and nutrient addition (Treatment IV).

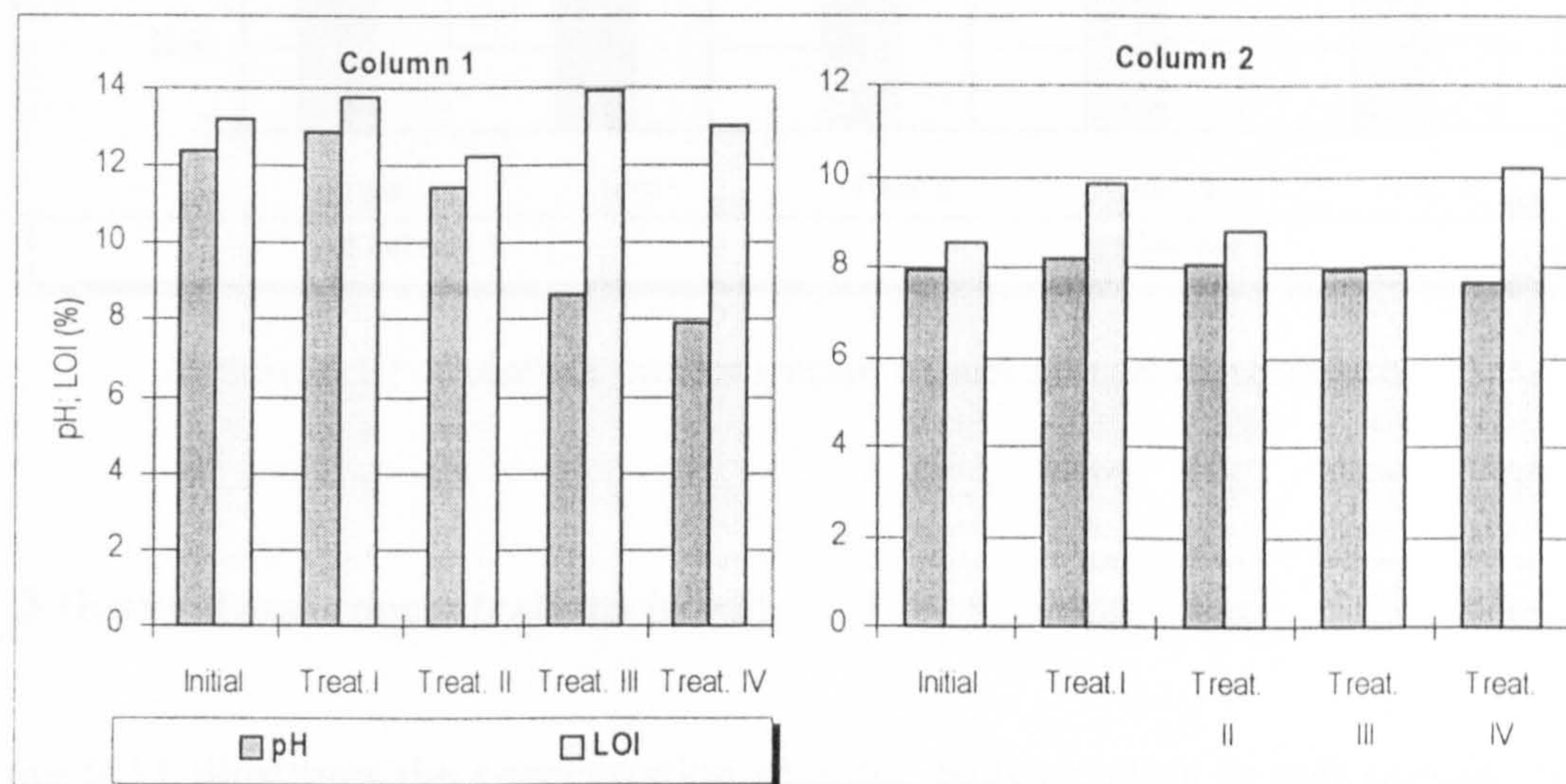


Figure 9.9 LOI and pH after each treatment in soil samples

9.3.2 Sulphate concentration in soil

The sulphate concentrations in the soil after treatment are illustrated in Figure 9.10 demonstrating clearly that the treatment given could reduce the sulphate concentration in the soil column. Both Columns 1 and 2 produced similar results. Sulphate was retained in the soil after the increase in deionised water addition (Treatment I) but subsequently reduced after aeration (Treatment II) and nutrient (Treatment III), and glucose+nutrient addition (Treatment IV).

In Column 2, Treatments III and IV resulted in similar amounts of sulphate being retained in the soil.

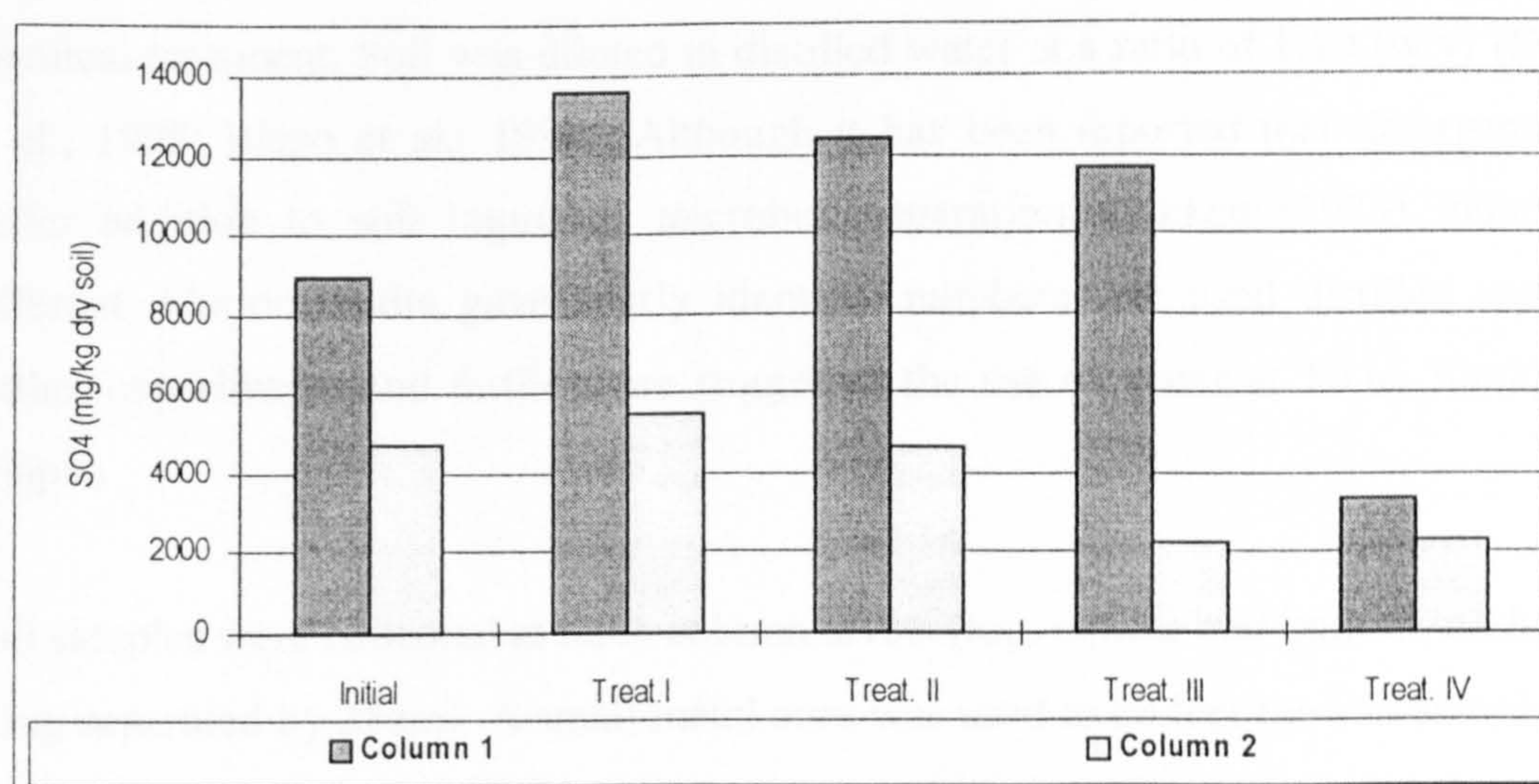


Figure 9.10 Sulphate concentration in soil after each treatment

9.3.3 Heavy metal concentrations in soil

Figure 9.11 illustrates the concentration of some heavy metals in soil samples taken from the columns. Only arsenic showed a reduction after treatment while other metals such as Zn, Mn, and Cu were not reduced. Cu even increased almost one hundredfold after glucose and nutrient addition (Treatment IV). Mn was slightly reduced after Treatment IV. These results were different from the heavy metals in the leachate where Zn and Mn were lower after glucose and nutrient addition. High copper leaching was probably related to glucose addition and the process in the soil after glucose addition.

9.3.4 Bacterial count in soil

The total bacterial count was estimated using the acridine orange direct count method after soil extraction in a shaker at 1500rpm for 20 min. Although, there was no significant improvement in this study, Tween 80 at 0.01% (or 10 μ g/ml) was used to increase the microbial separation from soil particles (DoE, 1991; Kepner and Pratt, 1994; Bakken, 1995; Yoon and Rosson, 1990; Allwood, 1971). The soil sample used for the microbial enumeration was only 0.5g, consequently the soil extraction could not be carried out in a way similar to those in the microbial growth tests. As suggested by Kepner and Pratt (1994), soil dispersion may be enhanced by physical and

chemical treatment. Soil was diluted in distilled water at a ratio of 1:10 (w/v) (Durant et al., 1995; Bispo et al., 1998). Although it has been reported that detergents and buffer addition to soil improved microbial separation, Bakken (1985) found that different dilution media gave nearly identical numbers. He used distilled water in further experiments and furthermore suggested the use of water at 10 to 50ml/g soil sample.

Soil samples were collected at three column levels (top, middle and bottom, each point being separated by 25cm). A small metal core was used to collect the soil samples but it was noted that the amount of soil collected was not always the same quantity between treatments or even between sampling ports. Sometimes, the soil was too wet to collect and only a small amount could be collected. However, at one port the soil samples were collected at three radial position with the objective of collecting a more representative soil sample. The samples were air dried prior to analysis.

Figure 9.12 shows the results of the total bacterial counts at each port after each treatment. In Column 1 higher bacterial counts were found after Treatments I and IV whereas in Column 2 this was only obtained after Treatment IV. The numbers of bacteria were also different between soil sample ports. In Column 1 after Treatments I and IV, the middle level was lower than the top or bottom sections. After nutrient addition, the bottom port showed the highest number. In Column 2, however, it was always seen that the bacterial count at the top was the highest.

The study also attempted to consider the morphology of the colonies found in the plate counts. During the experiment, some colonies always appeared on the plate including white, yellow and orange colonies. Gram staining of some is shown in Plate 9.1.

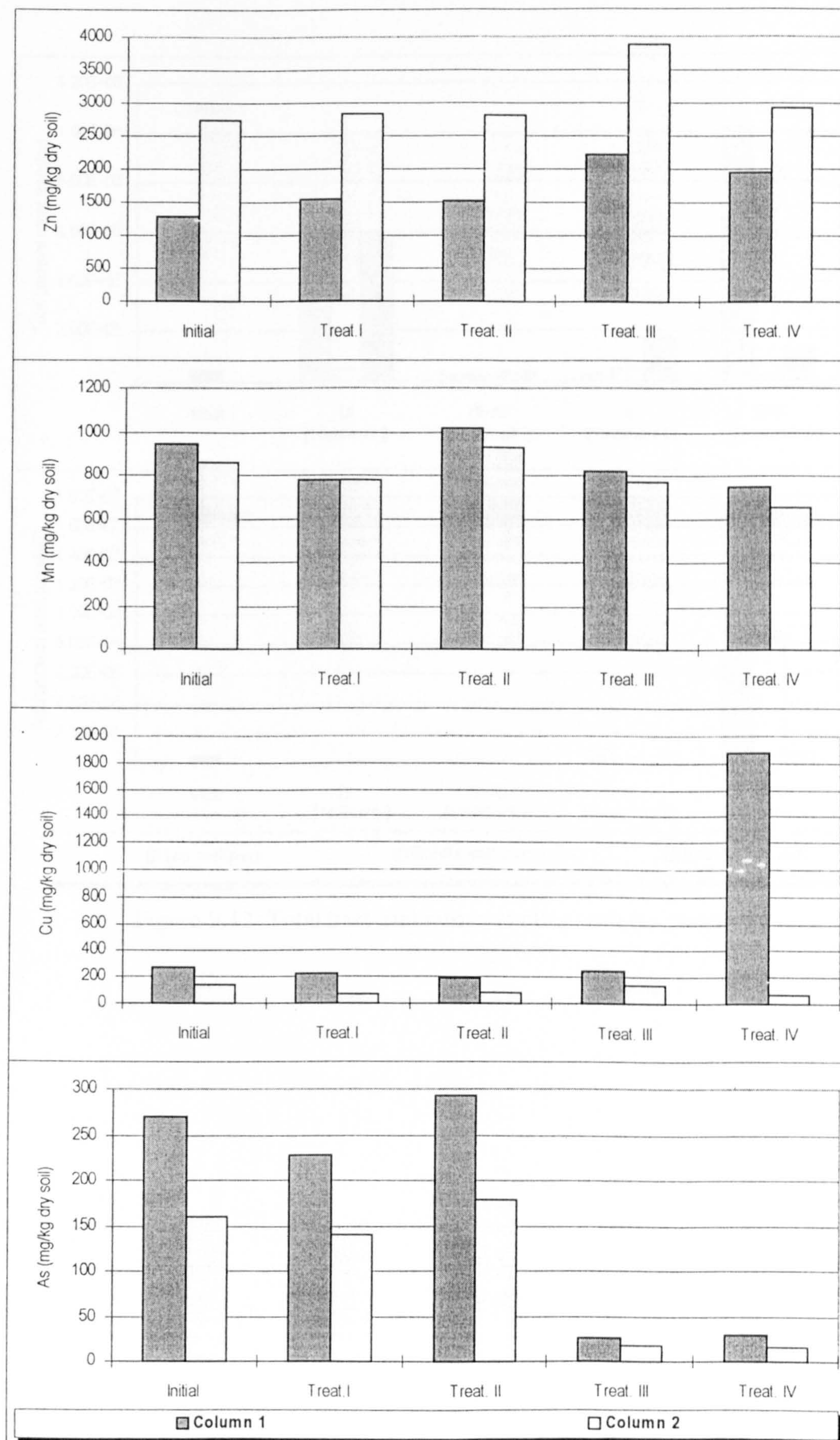


Figure 9.11 Heavy metal concentrations in soil after each treatment

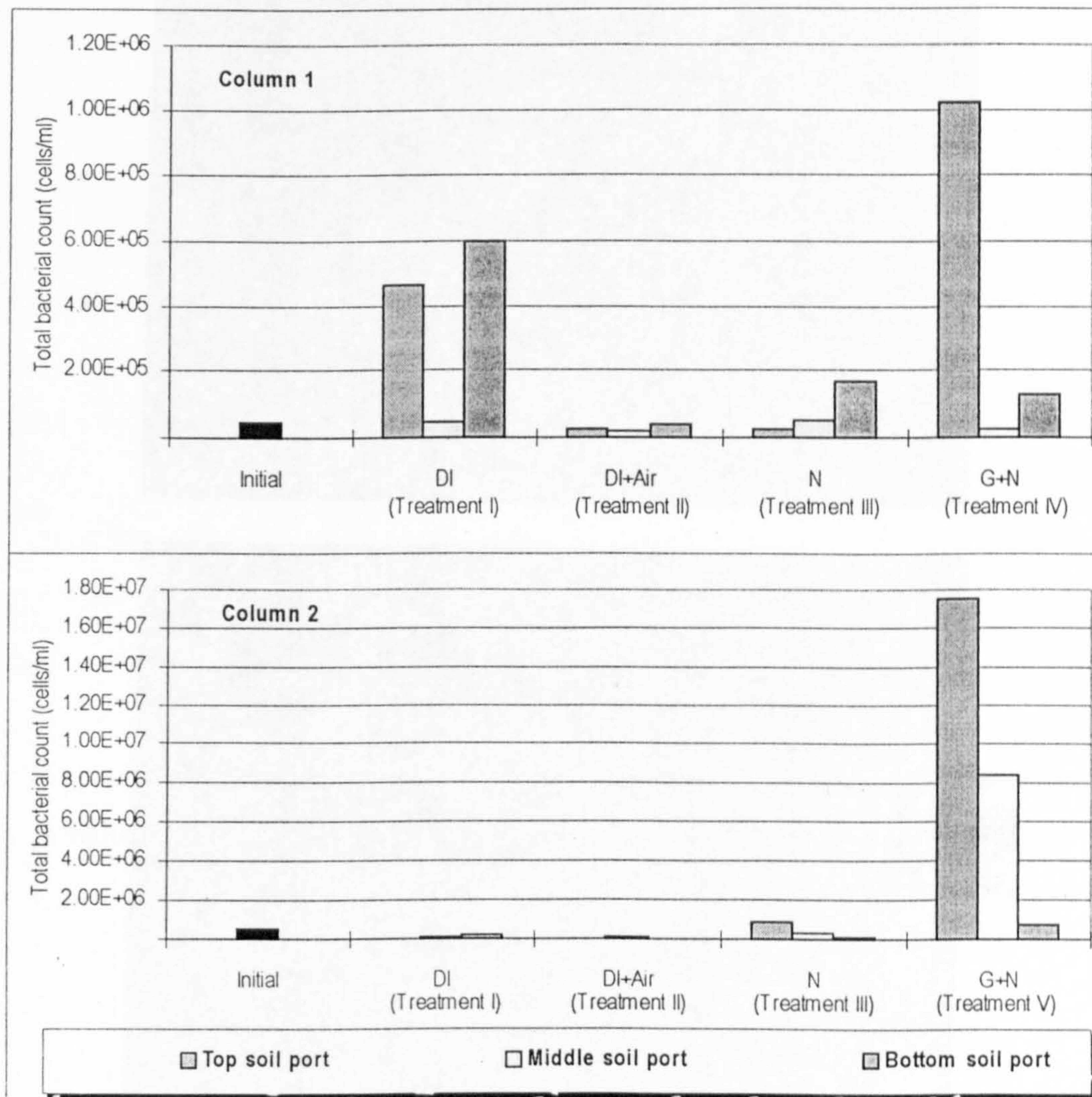


Figure 9.12 Total bacterial numbers after each treatment

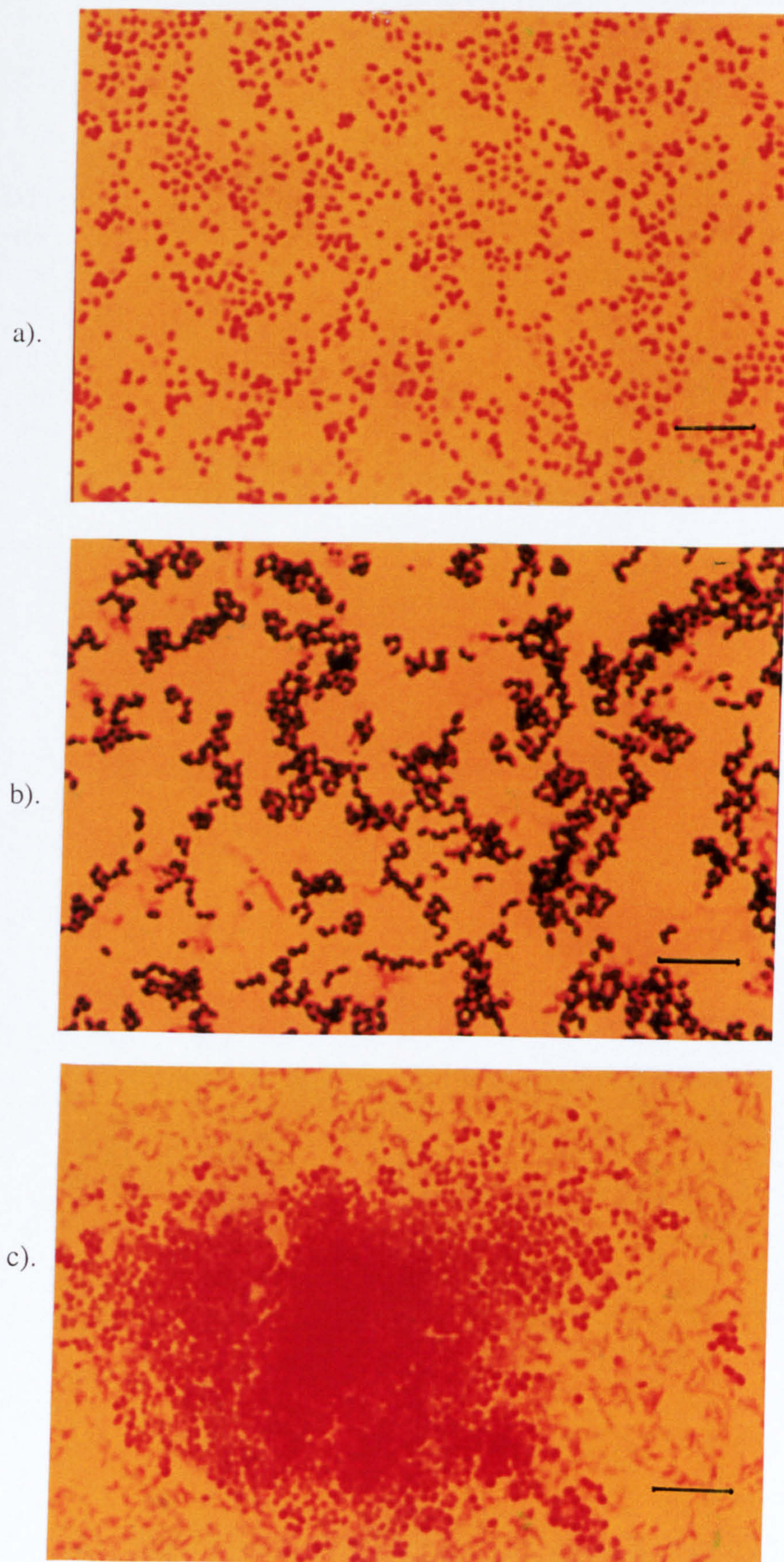


Plate 9.1 Microbial morphology of some colonies in a soil sample from the columns
(a). White colony, (b) Yellow colony; (c) Orange colony; Bar: 100 μ m

9.4 Discussion

Although treatment with aeration would not have been undertaken, based on the results of the slurry tests, the result of Treatment II would be included to confirm the appropriate condition for sulphate reduction in this study. In the Treatment II (deionised water and aeration) the leachate from the soil columns contained a slightly increased sulphate concentration on aeration. In the soil samples from the columns, however, sulphate was found to be lower than with deionised water addition alone (see Fig. 9.10). The aeration during the soil column test did not significantly change the concentration of Zn or Mn in the leachate. This was also shown in the soil of column samples for Zn and Cu. Manganese and arsenic, furthermore were present in their highest concentrations after Treatment II (Fig. 9.11).

With regard to CO₂ evolution, the column test showed that aeration increased the amount of CO₂ evolved. Aeration in Column 2 (Soil 5) produced even more CO₂ than for nutrient addition, as shown in Fig. 9.4. Although it may be assumed that microbial activity increased as the respiration rate increased, this should be confirmed by other evidence. Unfortunately, high CO₂ production was not supported by the microbial population data. Bacterial counts in soil from the Column 2 for Treatment II revealed that aeration did not increase the microbial population in the soil samples. The microbial population was in fact the lowest when compared to other treatments for both soil columns (see Figure 9.12). The LOI that was considered to represent organic matter was also lower than for Treatment I, which implied that there was no increase in biomass. This could be related to the heavy metal concentration in the soil samples that could have possibly affected the microbial population. The CO₂ production was higher but the microbial population required more energy for biomass synthesis (Chander and Brookes, 1991; Fließbach et al., 1994)

Those results favoured the suggestion that anaerobic conditions provided a more suitable environment for the soil studied. However, in Column 2 aeration increased CO₂ production compared to nutrient addition alone. One possibility causing this result was related to the moisture content and the aeration effect. Howard and Howard (1993) suggested that O₂ uptake or CO₂ evolution were low when the soil moisture

was only 5-10% of the water holding capacity (WHC). The maximum responses could be achieved at moisture content of 30-45% or 60% of the WHC for the soil samples they used. Beyond this point, the O₂ uptake decreased up to the WHC. They explained that at saturation point, microbial activity was depressed by poor aeration and reduced availability of oxygen. In Treatment II of Column 2 aeration increased CO₂ evolution as the porosity of Column 2 was greater than those in Column 1.

With regard to sulphate concentration, temperature and soil moisture content had been found to be the most influential factors for mineralization and immobilization rates of sulphur in soil (Zhao et al., 1996). The rate of mineralization increased exponentially at the beginning and diminished as biological optima were reached. Above the optimal temperature the responses became negative. Mineralization of soil organic sulphur is retarded at low levels and when approaching soil moisture saturation (Howard and Howard, 1993; Zhao et al., 1996). Furthermore, Zhao et al. (1996) stated that rewetting the soil after drying enhanced mineralization and produced a flush of sulphate. This was found to be so in this study when Treatment I (deionised water) was carried out, with sulphate being produced in high concentrations in the leachate over the first 3 days. They explained that flushing of sulphate was mainly due to decomposition of the microbial biomass killed during air-drying. The moisture content also affected the mineralization products. Under aerobic conditions the principal product was sulphate whereas under anaerobic conditions it was sulphide, elemental and volatile compounds (thiols) (Zhao et al., 1996). The soil column studied, under anaerobic conditions, did not show any H₂S production. This probably related with high pH of each soil (pH of 8 and 11) where the H₂S could be in low pressure. In addition, the column was operated as an open system which allowed leachate to drip throughout the experiment. Nonetheless, the soil became darker in colour and a sulphide odour was detected when dismantling the column. This could be evidence that sulphide was produced in the soil column.

9.4.1 Response to nutrient additions

After Treatment III (nutrient addition), the pH in the leachate from Column 1 (Soil 4) fell from around 12 to 9.4 whereas from Column 2, the pH did not significantly change. This was similarly shown for the soil samples from the columns. The pH in

soil Column 1 decreased to around 8.5 (from pH 11). Changes in pH of soil Column 2 were not so readily noticeable.

Nutrient 1 that was added at double strength in soil column experiment did not stimulate sulphate reduction. On the contrary, the sulphate concentration in the leachate collected increased considerably. This was found in both soil columns. However, the sulphate recovered and measured in the soil column samples was markedly reduced (see Fig. 9.10). Nutrient addition appeared to leach sulphate out of the soil column and left the soil with a lower sulphate content.

In the soil from the Columns, zinc increased significantly due to nutrient addition (Treatment III) but manganese was slightly reduced. The manganese in leachate of Column 2 was also present in lower concentration. Arsenic in soil of both Columns was significantly decreased after nutrient addition.

In the soil column tests, compared to Treatment II (deionised water+aeration), nutrient addition (Treatment III) increased CO₂ production in Column 1 (Soil 4) but were found to be lower in Column 2 (Soil 5). The LOI in the soil of Column 1 was the highest after nutrient addition (Treatment III) whereas in the soil in Column 2 it was the lowest. The bacterial numbers in soil Column 1 after nutrient addition were higher than in Column 2.

The addition of nutrients resulted in an increase in microbial population compared to the treatment with deionised water+aeration. This was found to be true for both soil columns. The difference was that of the location of microbial growth. In soil column 1, a high microbial population was found at the bottom of the column whereas in Column 2, the highest number occurred at the top.

The responses of nutrient addition to soil samples were important in changing the sulphate concentration, the heavy metals and the gas production. Nutrient addition decreased the pH and some heavy metals and CO₂ production but increased sulphate concentration. Arsenic concentration in the soil reduced significantly after nutrient addition. For Soil 4 (Column 4) nutrient addition could also significantly change the

pH, CO₂ production and the bacterial population. This suggested that soil types and characteristics very much affected the process and responses.

The results of the column test were considered to be closer to a contaminated soil situation in the field since the test was conducted in the solid phase. Its application however, would rely on further investigations on carbon source addition and other environmental considerations.

9.4.2 Response to carbon source addition

After glucose and carbon source addition, the pH of the leachate from Column 1 decreased by around 2 units whereas in Column 2 it was relatively unchanged. The soil from the columns showed that the pH after Treatment IV (glucose+nutrient addition) also reduced. The reduction in pH in Column 1 was significant in that it decreased from around 12 in the initial soil sample to pH 8 after glucose addition. The pH of Soil 5, on the other hand, was not significantly decreased. It reduced to pH 7.69 from 8.23. The sulphate concentration was also found at a lower concentration in the leachate from both soil columns after Treatment IV which was similar to the sulphate concentration in the soil sample from the column. However, it was not the case in the slurry phase as the sulphate was found to be higher in both Soil 4 and Soil 5.

In the soil samples of the columns, glucose and nutrient addition did not reduce Zn or Mn. In addition, copper was found at a very high concentration. The arsenic content was also unchanged after glucose addition since it was markedly decreased by nutrient addition.

The CO₂ evolution results from glucose addition showed significant increases for both soil columns. This was accompanied by marked increases in bacterial populations of both soil samples.

Furthermore, the sulphate concentration, both in the leachate and in soil samples from the column were found to be significantly reduced. Zn in the leachate was relatively low for both soils, but the Mn existed at a higher concentration in Column 1. This showed that a reduction in sulphate could be obtained with a glucose and nutrient

combination. This agreed with previous results in which net-immobilization of organic sulphur was found after amendment of the carbon source and the inorganic nitrogen (Zhao et al., 1996). However, this present study showed that the soil studied also required a phosphorous supply which was evident from the results of the microbial growth and slurry tests.

Glucose was added at 2500µg/g soil as recommended by Wu et al. (1995) in the closed system. Although many investigators reported that with an increase in glucose, or carbon source, the carbon to sulphur ratio could increase the sulphur immobilization, although this study did not prove this theory. Salami and Anderson (1998) noted that an increase in glucose level did not produce a lower sulphate concentration in slurry tests.

Sulphate that was very mobile under aerobic conditions could be converted into biomass-sulphur, soil organic-sulphur or remain as sulphate. A study by O'Donnell et al. (1994) on the amendment of sulphate-S in arable soil together with the addition 2000 µg C / g soil in a closed system showed that 21-34% of the added sulphate was immobilised in 3 days. A similar proportion was converted into microbial biomass over the same period. Over 127 days there was little effect on sulphate and soil biomass-S. Between 3 and 10 days the total biomass decreased as a result of conversion into soil organic-S. Wu et al.(1993) used plant residue such as barley straw to amend the sulphate source. These studies, carried out in a closed system were different from soil column used for this experiment. They employed fumigation in a vacuum dessicator to analyse biomass production. In an open systems, such as soil columns, substances would be leached from the reactor. The amount of sulphur mineralised in an open system were generally greater than those in a closed system in which soil was incubated without leaching (Zhao et al., 1996). It was further explained that consecutive leaching could result in unusual physico-chemical conditions such as greater water content, pH changes, pore-size distribution and loss of nutrients. In an open system, the amount of sulphate produced in the soil has been shown to increase linearly with incubation time, indicating the mineralization rate. After some time the rate declined, however this period varied from soil to soil.

A study was carried out by White and Gadd (1996) with similar objectives as for this study but exploring more acidic contaminated soil. They utilised batch and continuous cultures for sulphate reducing reactors instead of soil columns and compared the use of carbon sources for bacterial sulphate reduction in order to remove sulphate, acidity, and toxic metals. They found that in batch cultures, lactate produced the greatest biomass, ethanol was efficient for stimulating sulphide production whereas acetate was less effective. In continuous cultures, ethanol and lactate were used directly as effective substrates while acetate resulted in slow growth. They criticized the use of glucose since it possessed a deleterious effect on pH due to its fermentation generated organic acids. Ethanol was also used by Barnes et al. (1994). White and Gadd (1996) study also covered a range of nutrient types used for the treatment. They found that cornsteep could be utilised as the nitrogen source and concluded that the treatment should combine ethanol as the carbon source and cornsteep as the complex nitrogen source. White and Gadd (1997) were also able to precipitate metals such as Cd, Cr, Cu, Ni, and Zn in an acid leachate in a sulphide-bioprecipitation system. They improved the biomass feedback by cationic polymer flocculant addition in the bioreactor with ethanol as the carbon substrate and cornsteep as the complex nitrogen source.

For soil remediation, it was preferable to use a soil slurry reactor rather than a 'packed-bed', or 'dry', soil column as it was easier and better controlled. Koning et al. (1998) compared both types and confirmed that when using a 'dry' system the time required was longer. In the slurry phase microbial conversion was enhanced. On the other hand, a column or 'dry' system would, in reality, be more likely be closer to contaminated soil. In practice, it was not easy to distribute additional substances since soil characteristics could be very heterogeneous and clogging of the soil pores by microbial growth could possibly have occurred. Some effort has been made to overcome this problem including the use of pulse injection to reduce the clogging by the microbial population. If the column system could perform well as a reactor, it would indicate that the microbial population had been growing satisfactorily.

9.5 Conclusions

Comparison of the results above led to the conclusions that the soils studied, which were classified as silty, with a high concentration of sulphate and heavy metals, could benefit from bioremediation. The column tests showed that sulphate could be reduced with the addition of glucose and nutrients. Zinc, manganese and copper could be removed by microbial-leaching and reduced with further treatment. Arsenic could be removed from the soil and reduced in the leachate by nutrient addition and reduced further with more glucose addition in Soil 5 while in Soil 4 nutrient addition alone was better. In addition, the soil column results also showed that the microbial population in the soil could increase with glucose and nutrient addition. This suggested that bioremediation of the sulphate and heavy metals in the soil studied did take place.

These conclusions suggest similar methods to remediate heavy metals from contaminated soil by utilising sulphate reducing bacteria as heavy metals were leached. The process requires carbon, nitrogen and phosphorous sources. With regards to the result of a similar study in bioreactors (White and Gadd, 1996), it was also found to be important to carry out experiments which covered a wider range of carbon, nitrogen and phosphorous sources. This should give greater benefits for the design of the treatment system in field.

PULL-OUT EXPLANATORY NOTE

SOIL SLURRY TESTS - METHOD I

Soil samples	Flasks	Nutrient	Amount of nutrient
Soil 1 (Contaminated)	Flask 1: (Control) Aeration only	N1 (N and P sources)	i). 'Once supplied': 2.5ml N1 added at 0 hrs
Soil 2 (Contaminated)	Flask 2: Aeration + Nutrient 1 addition		ii). '2.5ml' addition: 2.5ml N1 added daily
Soil 3 (Uncontaminated)	Flask 3: Aeration+ Act. sludge+Nutrient 1 addition		iii). '5ml' addition: 5ml N1 added daily
	Flask 4: Act.sludge+ Nutrient 1 addition, without aeration		iv). '10ml' addition: 10ml N1 added daily

SOIL SLURRY TESTS - METHOD II

Soil samples	Treatment settings	Nutrient types
Soil 1 (Contaminated soil)	I : Shaking in orbital shaker at room temperature	No nutrient
Soil 2 (Contaminated soil)	II : Shaking in water bath (36°C)	N1 (N and P source)
Soil 3 (Uncontaminated soil)	III: Shaking in water bath (36°C) + SRB sludge	N2 (N only)
		N4 (N, P and trace elements)

SOIL SLURRY TESTS - METHOD III

Soil samples	Treatment conditions	Nutrient types	Nutrient levels
Soil 4 (contaminated) Soil 5 (contaminated)	Aerobic and anaerobic	No nutrient; N1 (N+P sources) N2 (N only) N3 (P only) N4 (N, P, trace elements)	0.5N1; N1; 2N1 2N2; 10N2; 20N2 5N3; 10N3; 20N3 5N4; 10N4; 20N4
	Anaerobic + glucose at G1	No nutrient; N1 (N+P sources) N2 (N only); N3 (P only) N4 (N, P, trace elements)	N1; 2N2; 10N3; 10N4
Acidified soil samples	Anaerobic +glucose at G1	No nutrient; N1 (N+P sources) N2 (N only); N3 (P only) N4 (N, P, trace elements)	N1; 2N2; 10N3; 10N4
Normal and acidified soil samples	Anaerobic+glucose levels G1,G2,G3 (G1=low;G2=medium; G3=high)	N1 only (N+P sources)	N1

MICROBIAL GROWTH TEST

Soil samples	Treatment conditions	Nutrient types	Nutrient levels
Soil 4 (contaminated) Soil 5 (contaminated)	Soil extract	N1 (N+P sources) N2 (N only) N3 (P only) N4 (N, P, trace elements)	0.5N1; N1; 2N1 2N2; 10N2; 20N2 5N3; 10N3; 20N3 5N4; 10N4; 20N4
	Soil extract + glucose	No nutrient; N1 (N+P sources) N2 (N only); N3 (P only) N4 (N, P, trace elements)	N1; 2N2; 10N3; 10N4
	Acidified soil extract +glucose	No nutrient; N1 (N+P sources) N2 (N only); N3 (P only) N4 (N, P, trace elements)	N1; 2N2; 10N3; 10N4

SOIL COLUMN TEST

Soil samples	Treatment conditions	Amount added
Soil 4 (Column 1)	Treatment I: Deionised water addition	Column 1: app. 500 ml/day; Column 2: app. 125 ml/day
Soil 5 (Column 2)	Treatment II: Deionised water + aeration	Column 1: app. 500 ml/day; Column 2: app. 125 ml/day Air supply: app.2 l/min
	Treatment III: Nutrient 1 addition	Concentration: double strength of N1 (2N1)
	Treatment IV: Glucose + Nutrient 1	Glucose: 2500 µg/g soil

SOURCE OF NUTRIENTS (in mg/l of reactor volume)

N1	N2	N3	N4
N: NH ₄ HCO ₃ (10mg N/l) P: KH ₂ PO ₄ (5mg P/l) and K ₂ HPO ₄ (6mg P/l)	N: Urea at 10mg N/l	P: KH ₂ PO ₄ (5mg P/l) and K ₂ HPO ₄ (6mg P/l)	N+P+trace elements: Omex concentrate (1ml diluted in 10l)

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CHAPTER 10

RESULTS AND DISCUSSION OF LEACHATE TREATMENT USING REVERSE OSMOSIS

10.1 Experimental Programme

In this part of the study a low pressure reverse osmosis membrane (LPROM), which had a spiral wound sulphonated polysulphone membrane was used, having a maximum pressure capacity of 860 kPa (125psi). The study was aimed at investigating the use of the membrane to treat leachate from the Gateshead contaminated landfill site having a high sulphate concentration. There were two main studies involved namely (i) membrane fouling and (ii) pretreatment of leachate prior to the membrane application.

The first study investigated the effect of sulphate concentration and pressure on membrane fouling at a temperature of 30°C. The sulphate concentration was tested in the range of 10-15 mM SO_4^{2-} and pressures between 375-575 kPa. During the tests, an artificial leachate containing sulphate and metals such as zinc and chromium at 1.2mg/l and 0.26mg/l respectively was used. These concentrations were based on an initial study of the leachate quality by consultants investigating the site (ETC, 1995).

The pretreatment study involved a chemical process using coagulation and flocculation. The coagulants used in the experiments were ferric chloride, aluminium sulphate, barium chloride, and Zetag 92 (cationic polyelectrolyte).

10.2 Membrane Baseline Performance

Prior to any tests with the leachate, standardization of the membrane to be used was carried out. This involved rejection and flux of NaCl as the standard solution followed by determination of the permeate flux of distilled water which was then used as the reference prior to each run. Standardization (using 500 mg/l sodium chloride at 25°C and 414 kPa) of the Optimem RO2012-16 membrane is shown in Figure 10.1 The rejection of sodium was greater than 95%. The permeate flux was 3.5 l/h/m² or 1.65

l/h on average as illustrated in Figure 10.2. The flux of distilled water was lower than sodium chloride which was around 2.5 l/h/m² or 1.2 l/hr at 25% recovery. Prior to any run, the flux of deionised water was always obtained as a reference and as a measure of membrane cleaning performance.

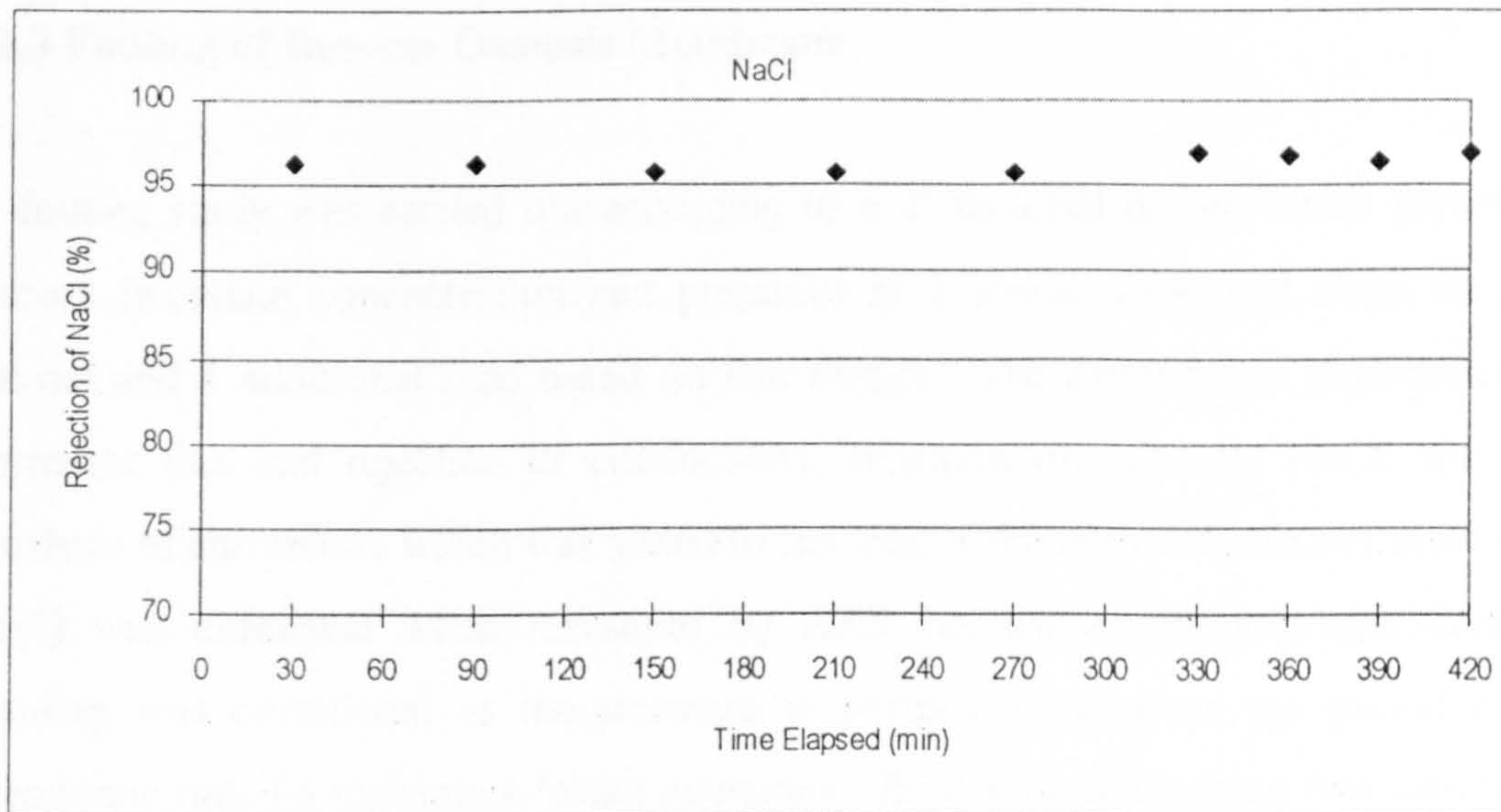


Figure 10.1 Rejection of Sodium (%) under standard conditions

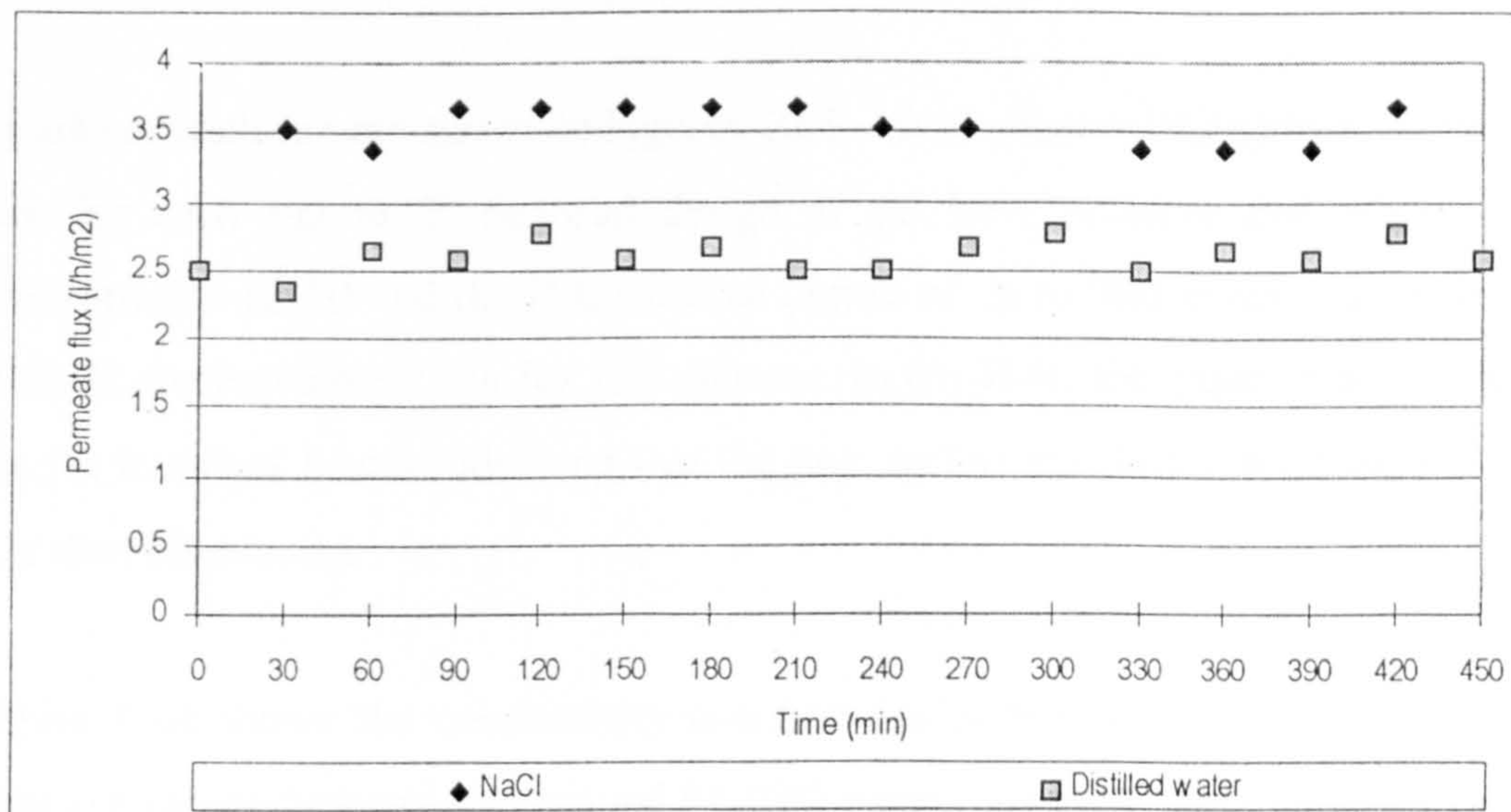


Figure 10.2 Flux of NaCl and distilled water under standard conditions

The permeate flux of NaCl solution was 3.5 l/h/m². This was considered to be lower than the flux obtained by Ujang (1996) which reached 4.0 l/h/m². The reason for the

different values was that the 2 membranes used in this study were considered by the manufacturer to be a slightly imperfect product. The flux obtained using a clean membrane was seen to be around 1 l/h. However, a third membrane performed better with a 1.55 l/h (3.33 l/h/m²) flux.

10.3 Fouling of Reverse Osmosis Membrane

A fouling study was carried out according to a 2² factorial design which involved 2 factors (sulphate concentration and pressure) at 2 levels (Low and High for each factor) and 5 additional runs based on star design. The experiments determined the permeate flux and rejection of conductivity, rejection of sulphate, NaCl, and zinc. Analysis of chromium, which was added to the feed at the reported concentration (0.26 mg/l) was unreliable when measured by AAS because of its low concentration. Fouling was considered as the decrease in permeate flux over the period of the membrane run. To maintain a 'clean membrane' for each run, cleaning was carried out after each run was completed. However, difficulty in washing and cleaning the membrane led to limitations in the time of each run. It was then decided to terminate a run if the flux decline reached at least 20%.

Results of each run are shown in Figures 10.3- 10.10. Figure 10.3 shows the permeate flux for each run in 2² factorial design at (a) Low pressure and High sulphate concentration (L-H) and (b) H-L for time period of up to 300 minutes (5 hours). For (c) L-L, the experiment ran for 150 minutes. In (d) H-H, the experiment was carried out for less than 1 hour indicating that the flux decline was faster and reached 20% in less than 60 minutes.

Figure 10.4 shows the conductivity rejection for each run. It can be seen that the rejection of conductivity was around 91-93% except for the H-H condition which was not completed because flux decline or membrane fouling had taken place before 1 hour of the run.

Figure 10.5 shows the rejection of sulphate, chloride and sodium. The membrane removed 98-99% of the sulphate during each run. Sodium removal declined over this

period, however, rejection of sodium was still high at 90-91%. Chloride rejection was similar to sodium except it was slightly lower in the L-L condition with only up to 88% rejection. Again, for H-H condition, only up to 90% rejection for sodium and chloride was seen over the 1 hour membrane run.

Rejection of zinc is presented in Figure 10.6 in which it can be seen that there were differences in response for each condition. The highest rejection was found in the H-L condition (80-86%) while the L-H still gave satisfactory results at 80% rejection. The lowest rejection was found for the L-L condition with only 55% at the beginning, increasing up to 70% removal. Zinc rejection in the H-H condition was around 77% after 60 minutes.

The five additional runs in the star design were suggested by Ujang (1996) and were aimed at investigating the central pressure and concentration effects on membrane performance. These results are shown in Figures 10.7 -10.10. The times of the runs were generally shorter than those of the first 4 runs. Only at M-LL condition did the flux decline by more than 20% after 300 minutes. Others were usually less than 150 minutes, even in LL-M the flux decline exceeded 20% before 1 hour.

Conductivity rejection in HH-M was the highest, at about 95%. Others were as low as 87% as shown in M-HH and LL-M conditions. The rejections at these two settings fell drastically with time (Figure 10.8). Sulphate rejections in four settings were high with almost 99% sulphate being rejected. However, under LL-M condition the sulphate rejection was at around 93% after 1 hour run operation. Sodium and chloride rejections were lower than sulphate which was around 94%. For both M-LL and LL-M, sodium was only rejected at 85% (Figure 10.9). Different results were found in zinc removal at M-LL and LL-M. Zinc had a low rejection at the beginning but it then increased slowly (Figure 10.10). However, at other settings the performance was at either steady state or it fell to only 56% for the M-HH condition and around 68% for the M-M condition.

The rejection results for each parameter in each run are described in Table 10.1 for 1 hour run, and Table 10.2 for the results after the completed runs.

Figure 10.3 Flux vs. Time

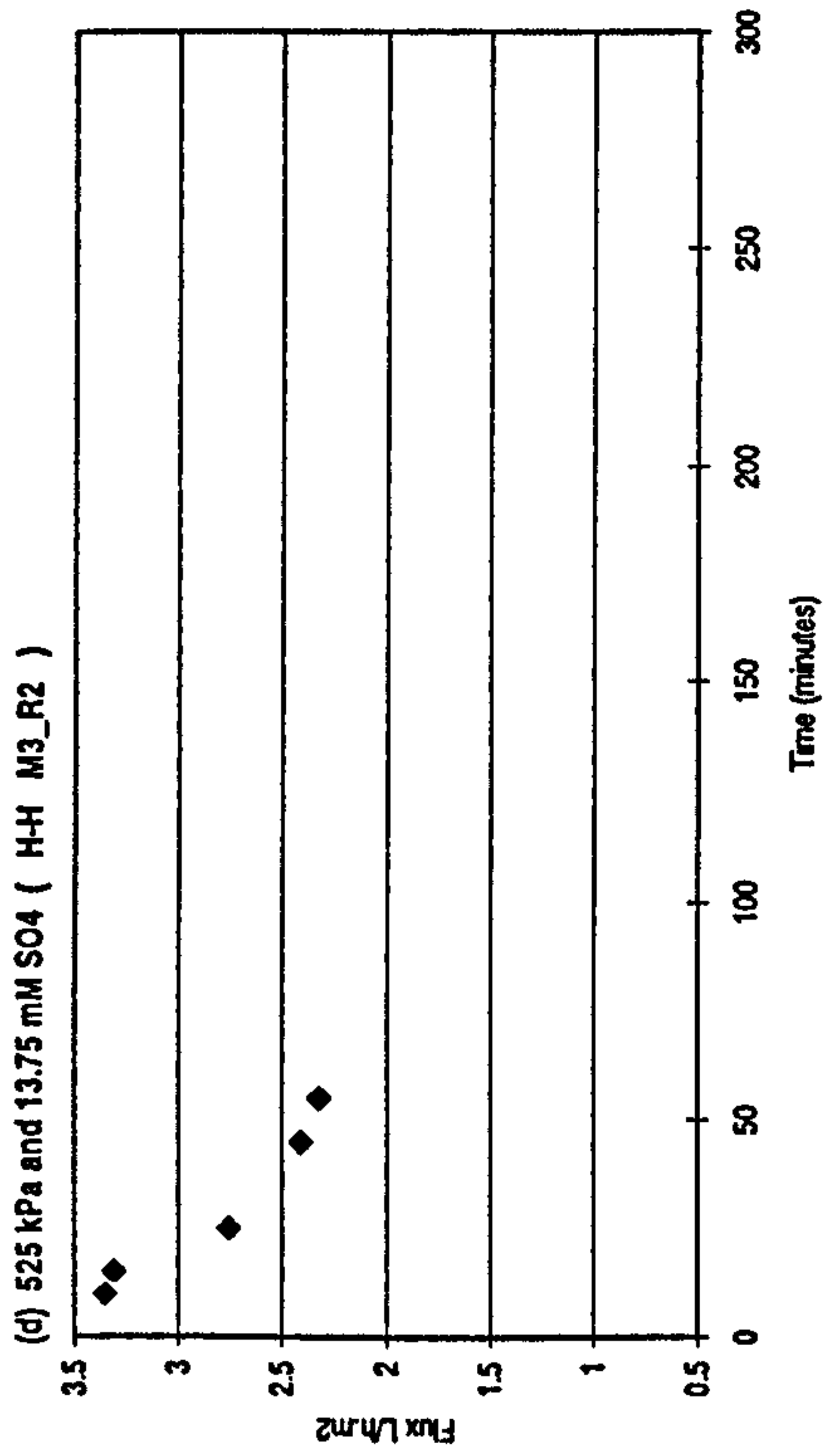
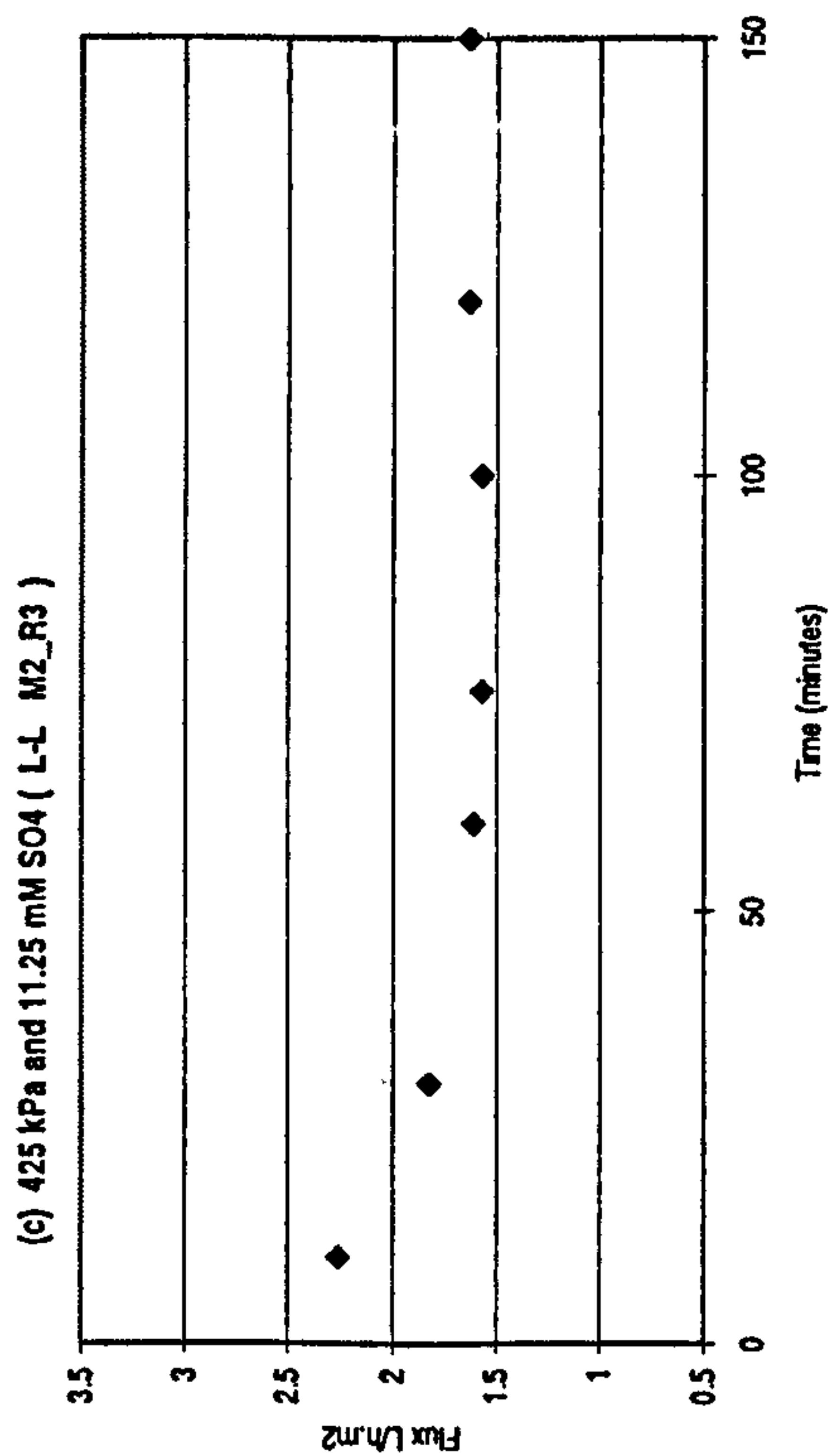
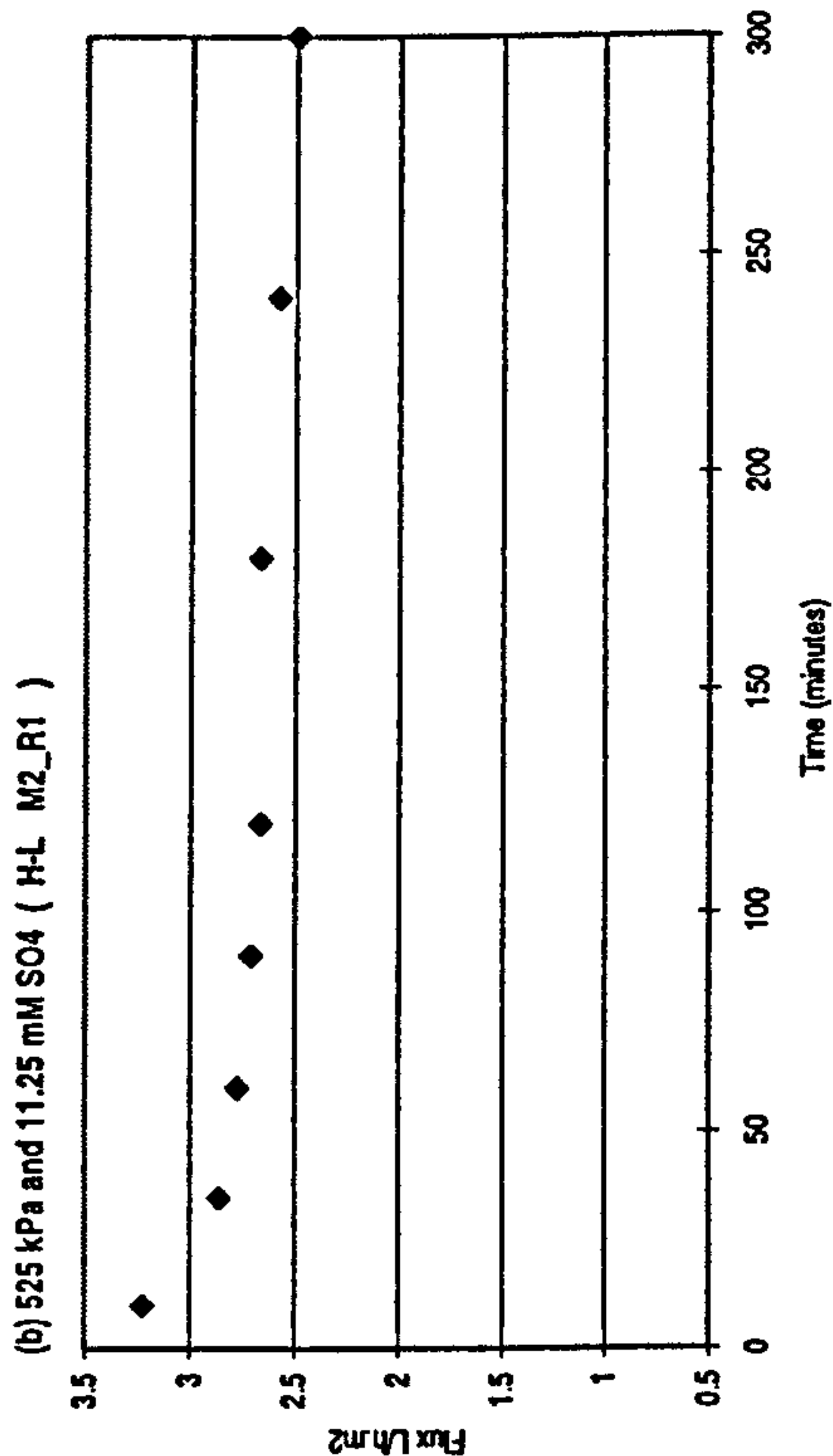
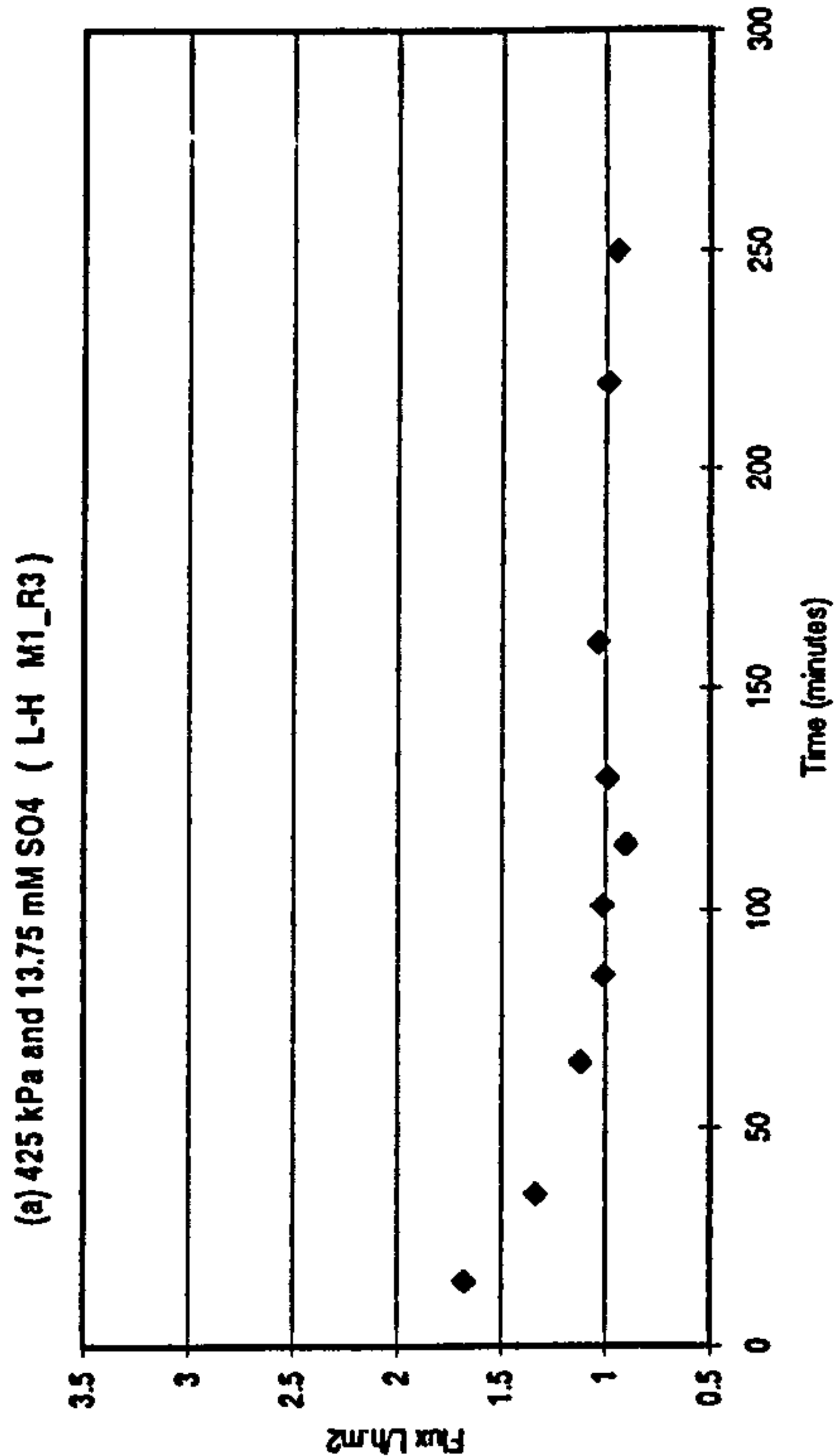


Figure 10.4 Conductivity rejection (%) vs. Time

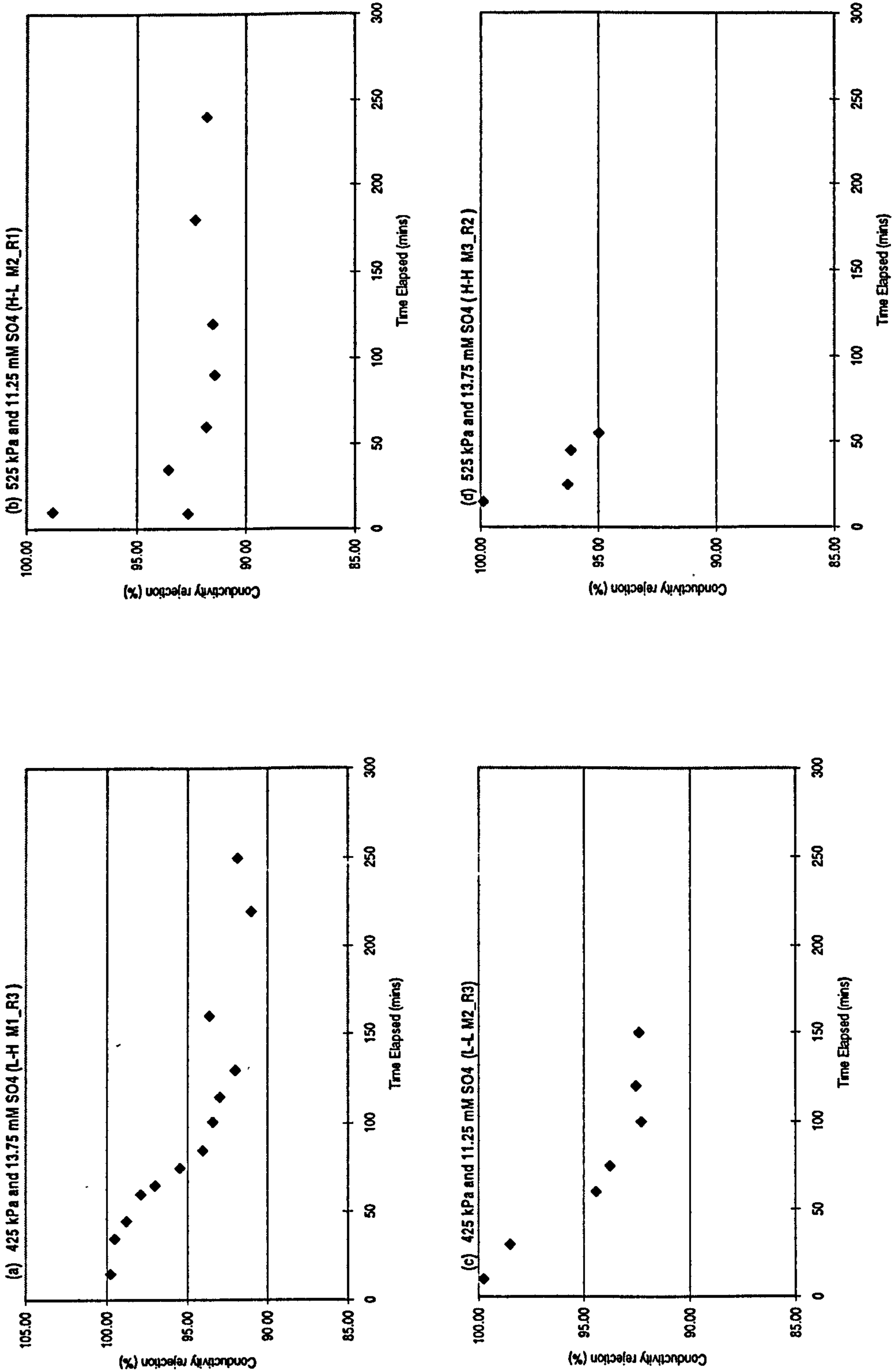


Figure 10.5 Sulphate, Chloride, and Sodium Rejection (%) vs. Time

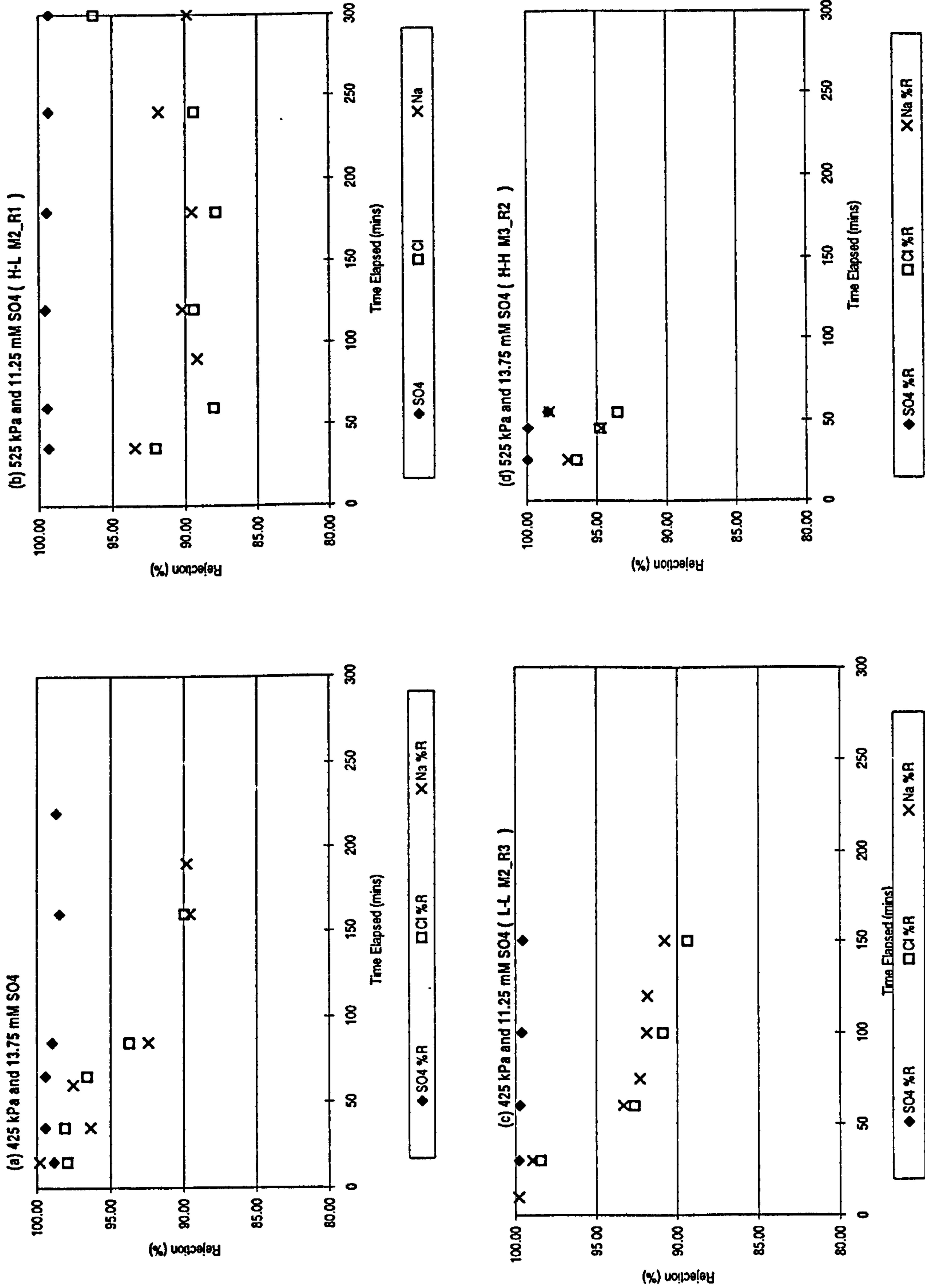


Figure 10.6 Zinc Rejection (%) vs. Time

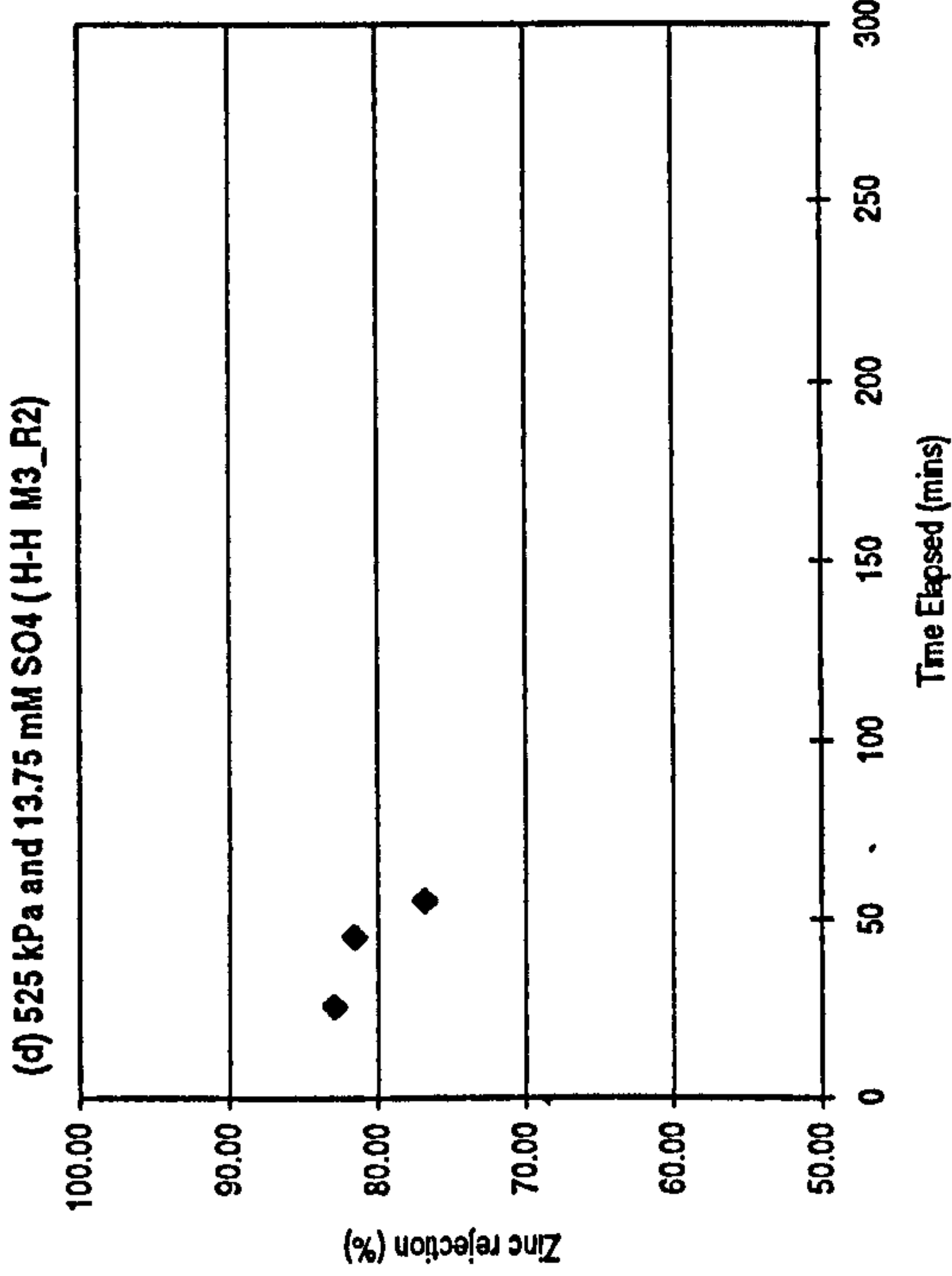
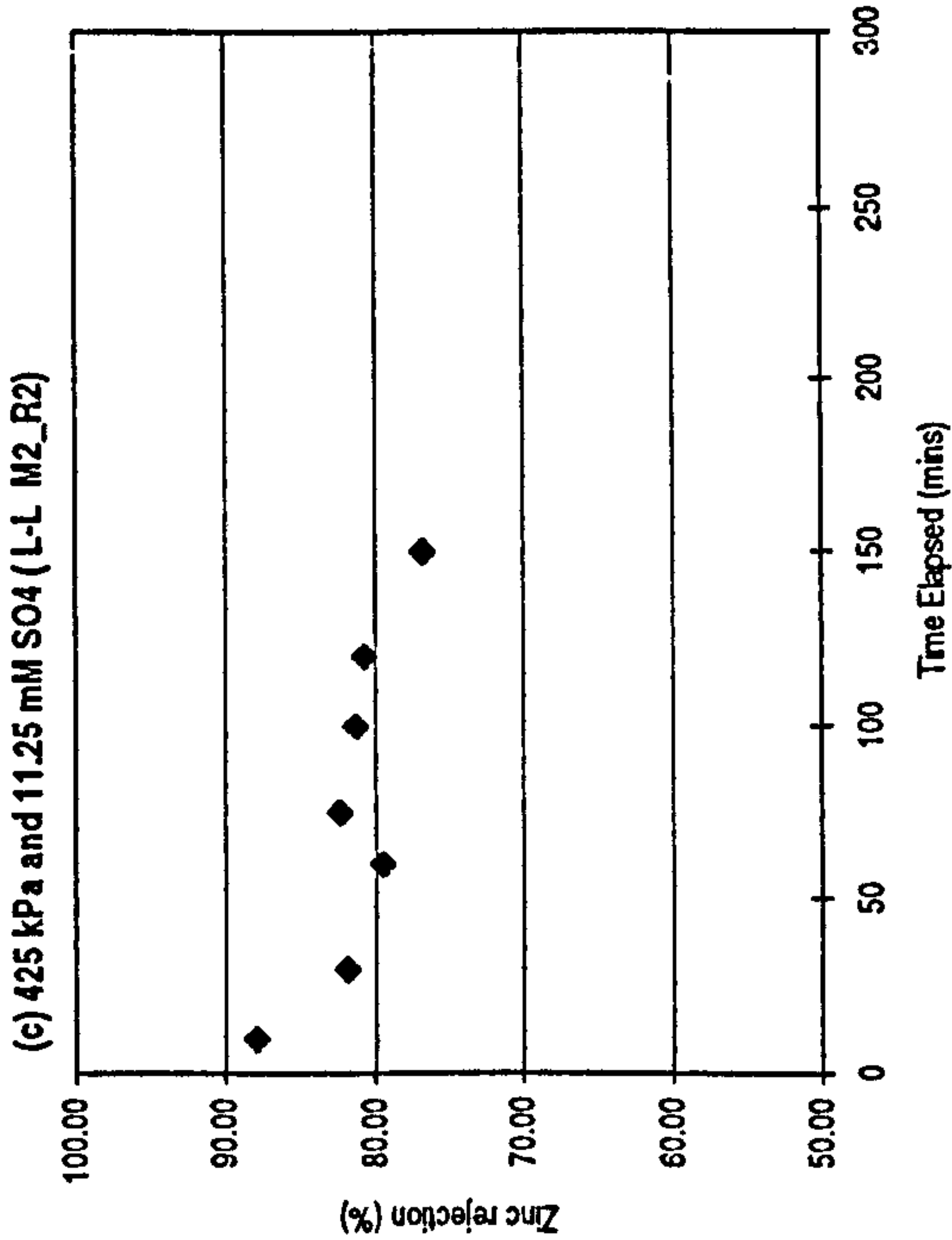
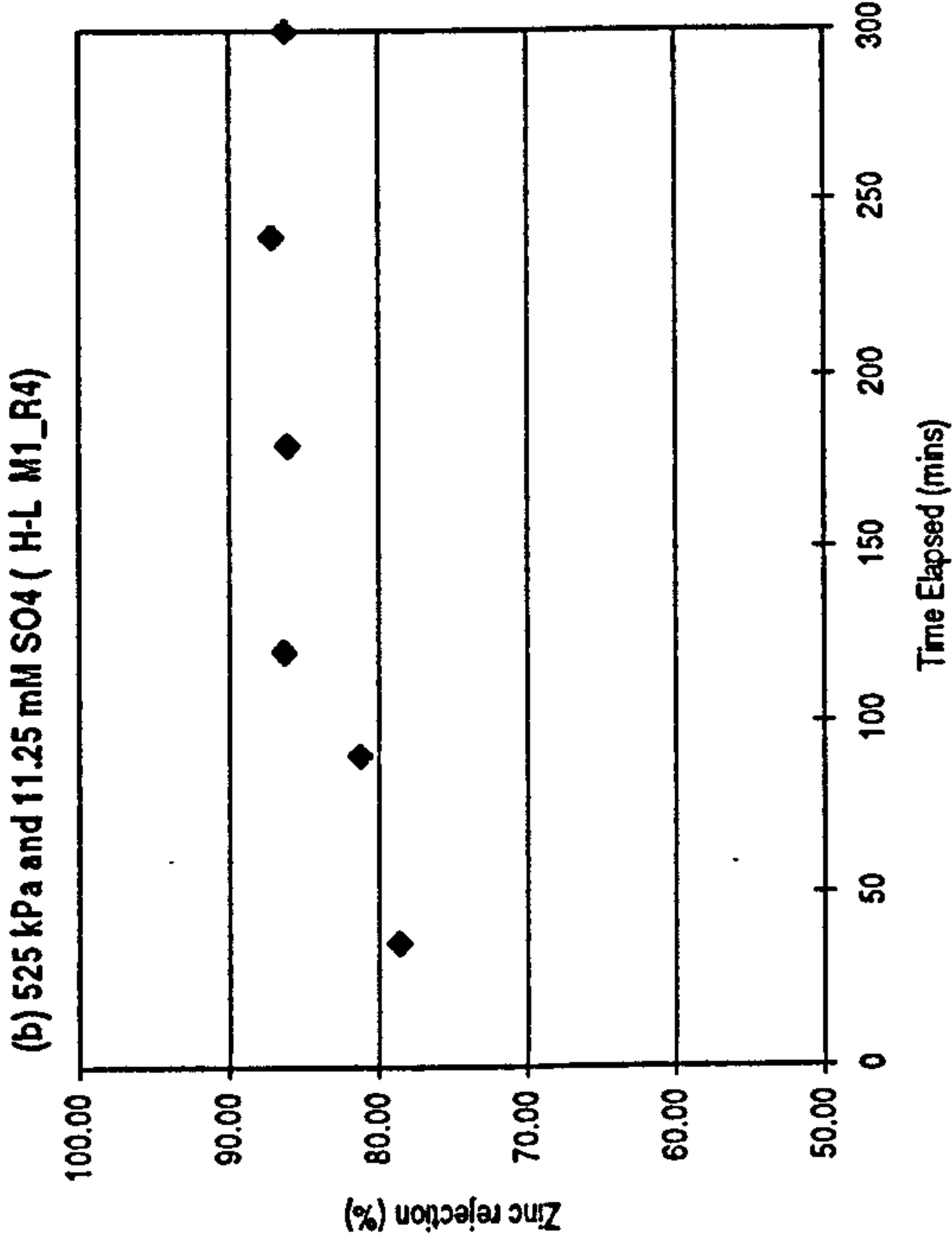
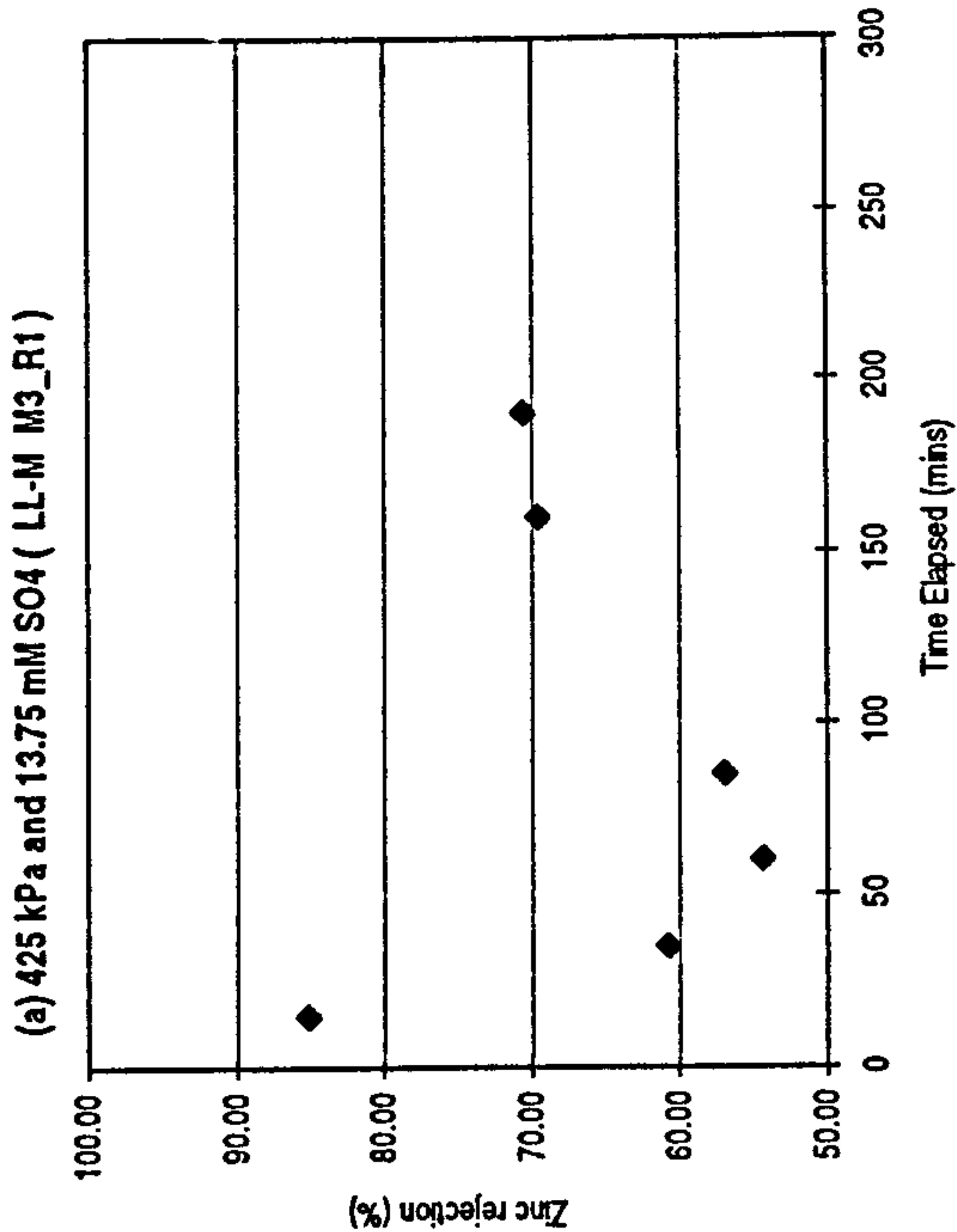


Figure 10.7 Flux vs.Time

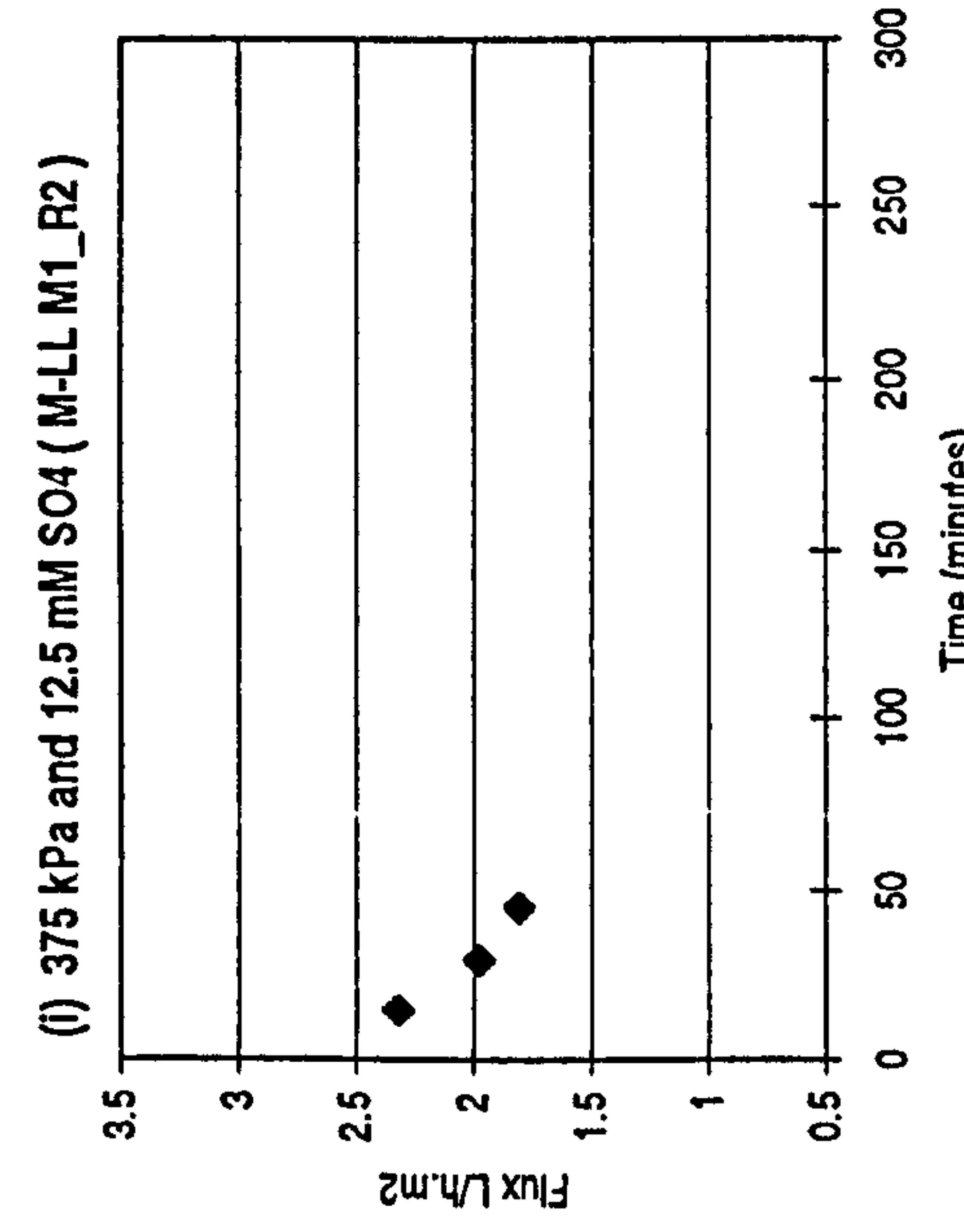
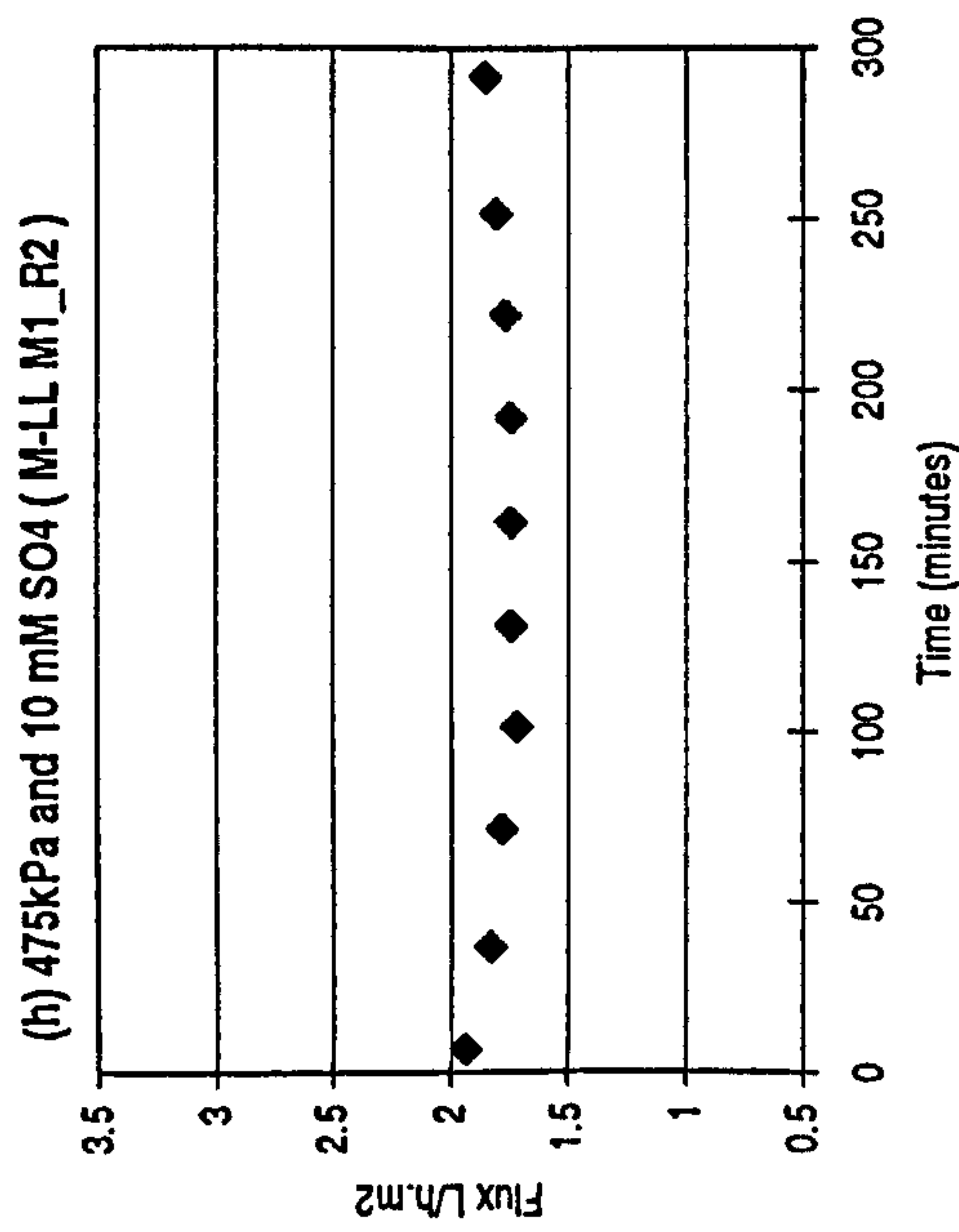
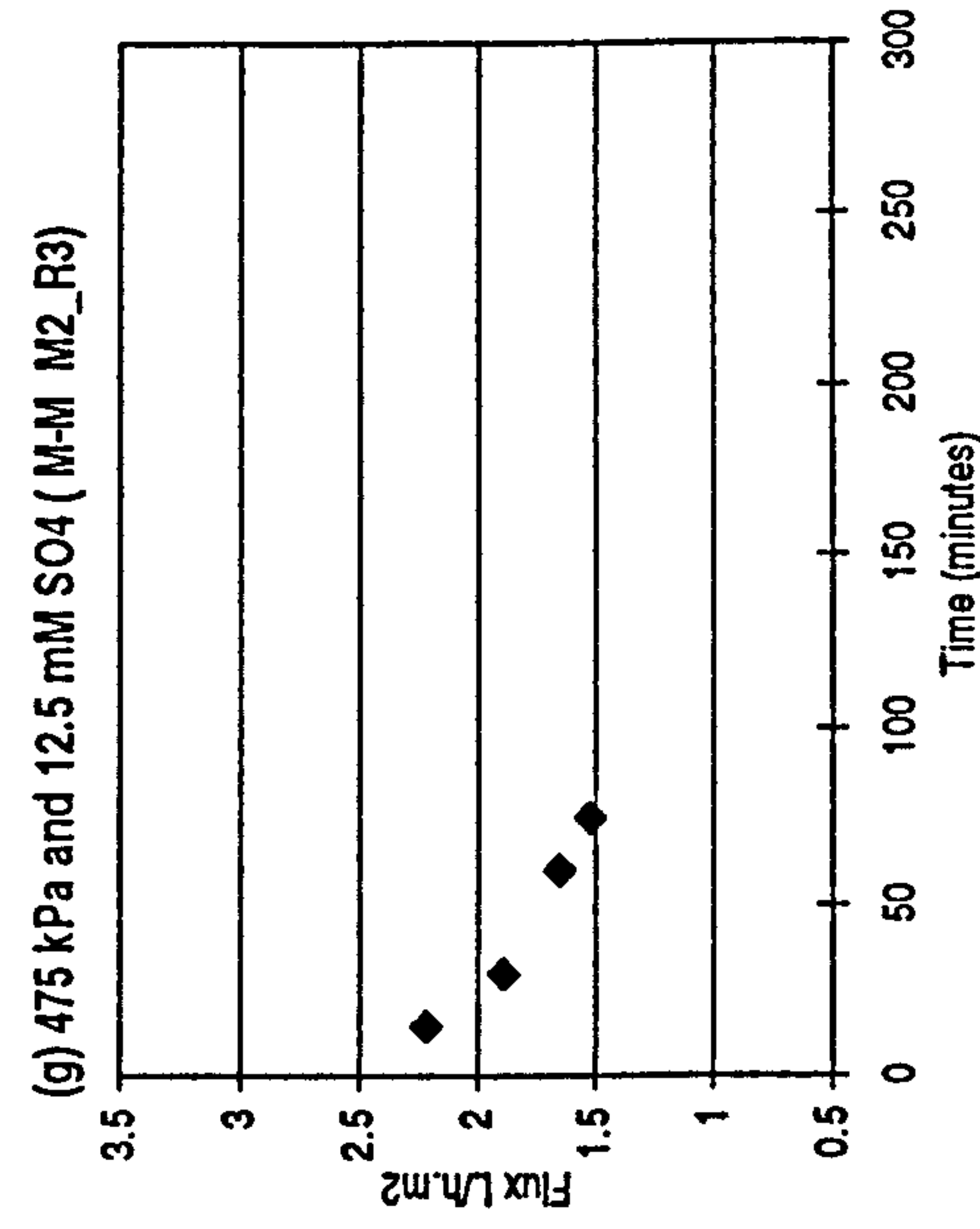
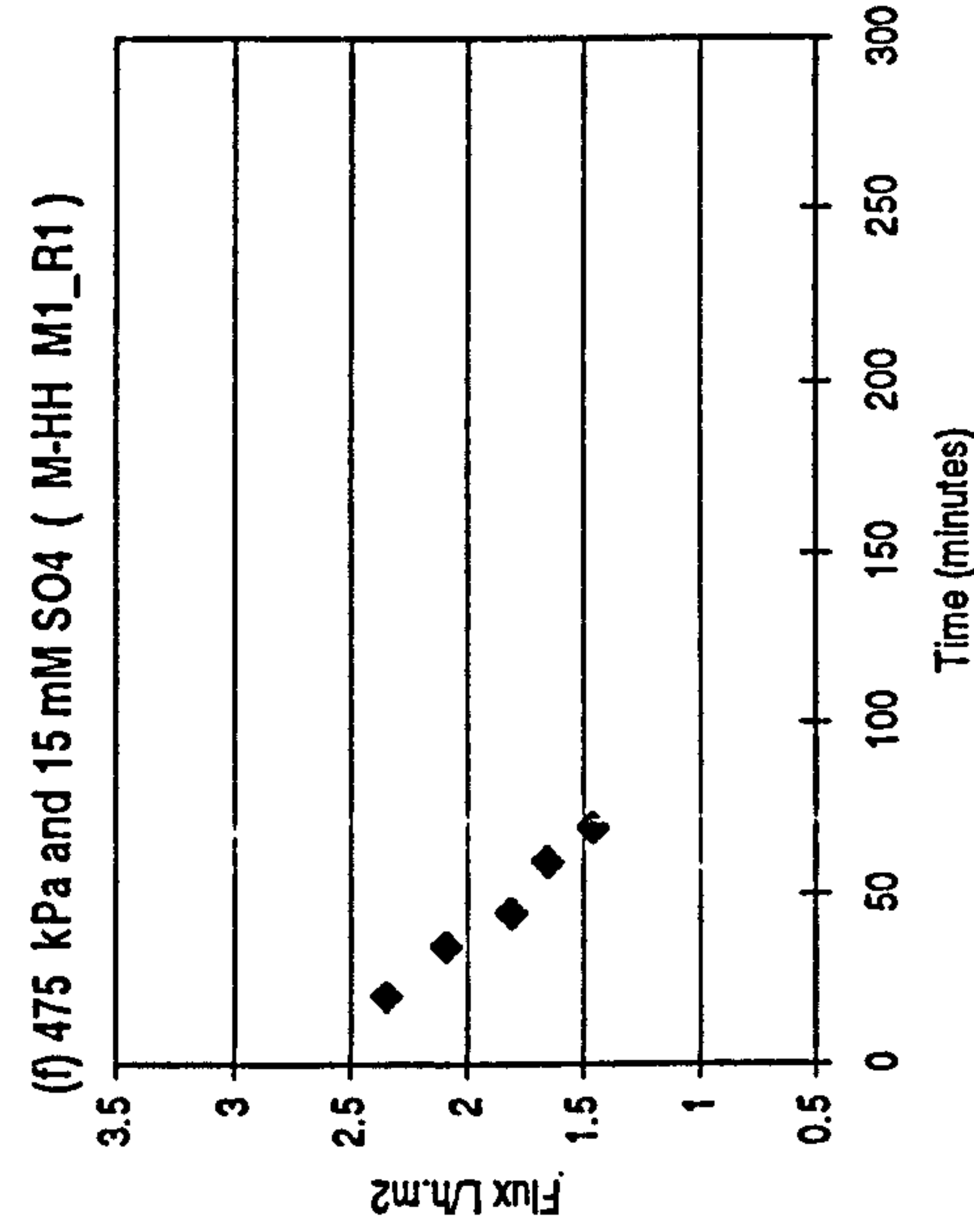
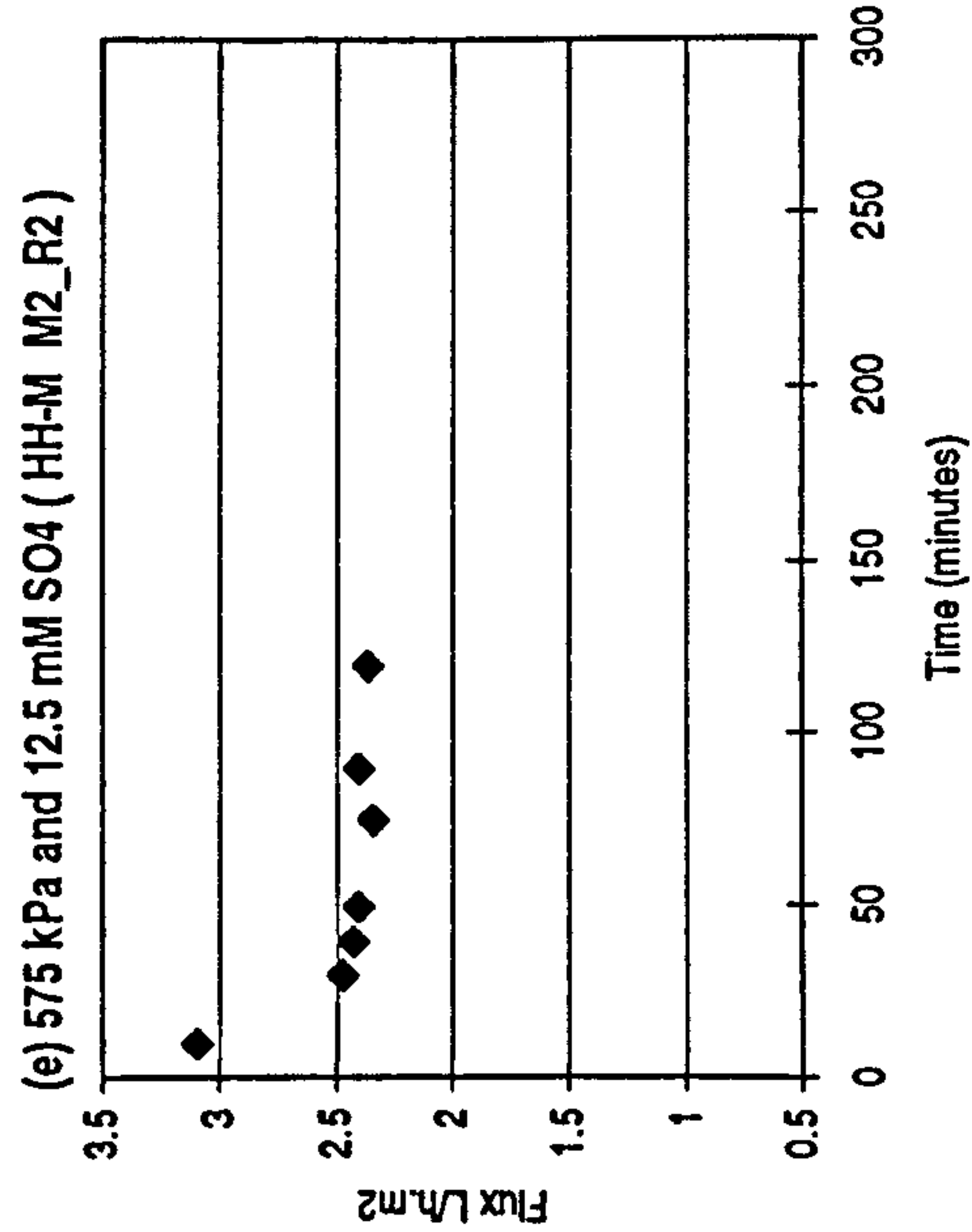


Figure 10.8 Conductivity rejection (%) vs. Time

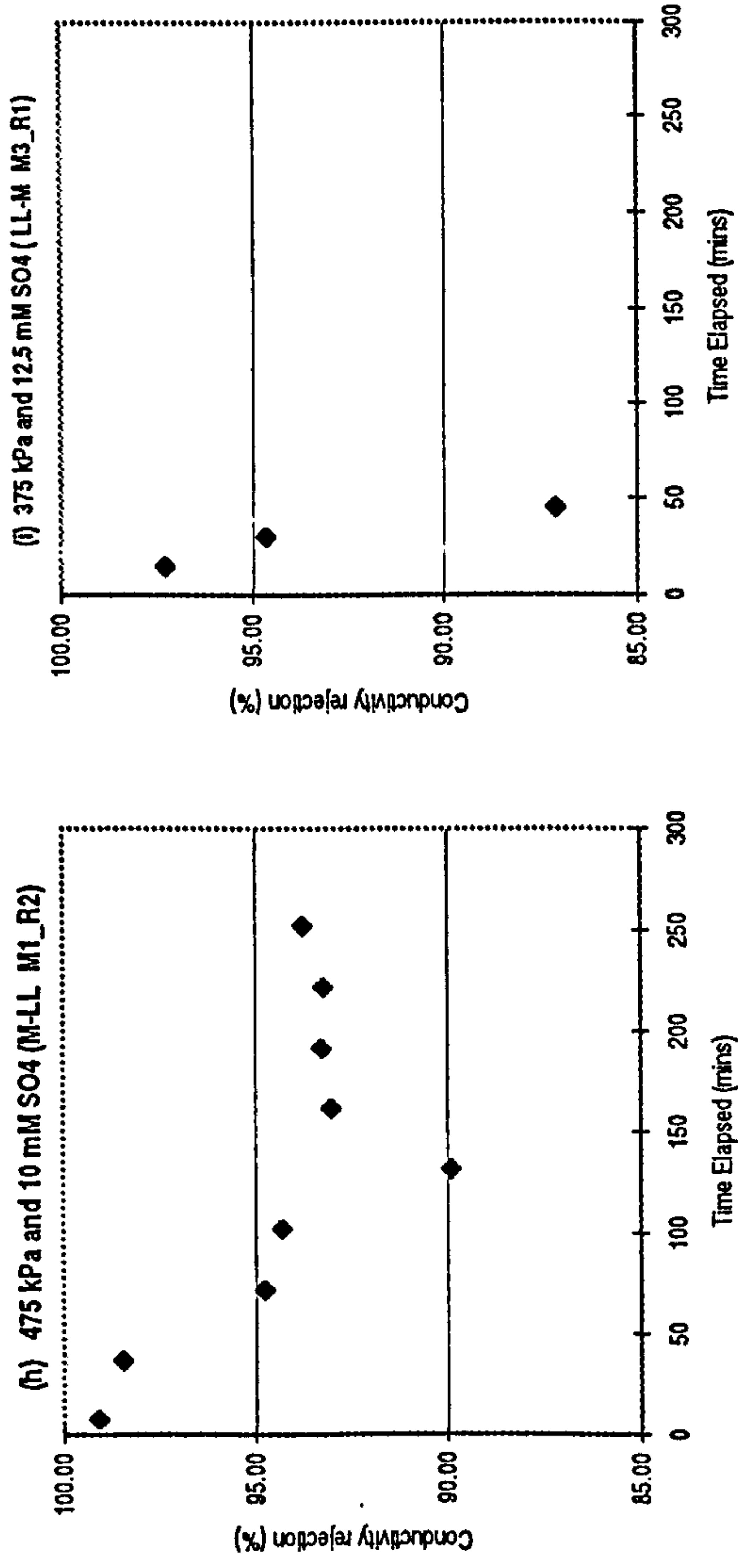
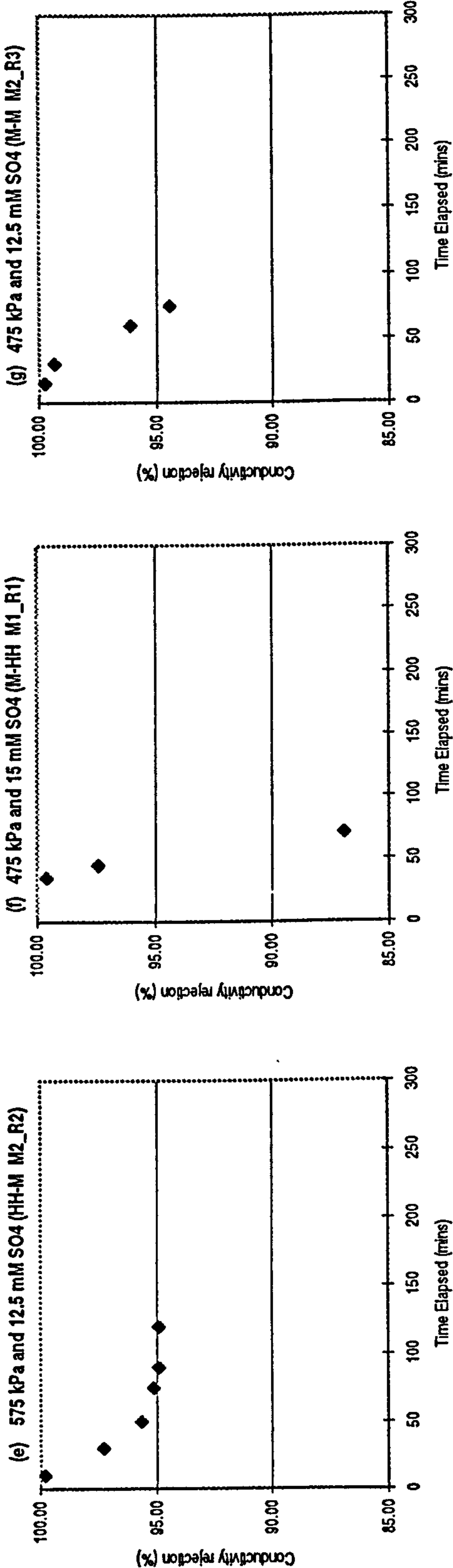


Figure 10.9 Sulphate, Chloride, and Sodium Rejection (%) vs.Time

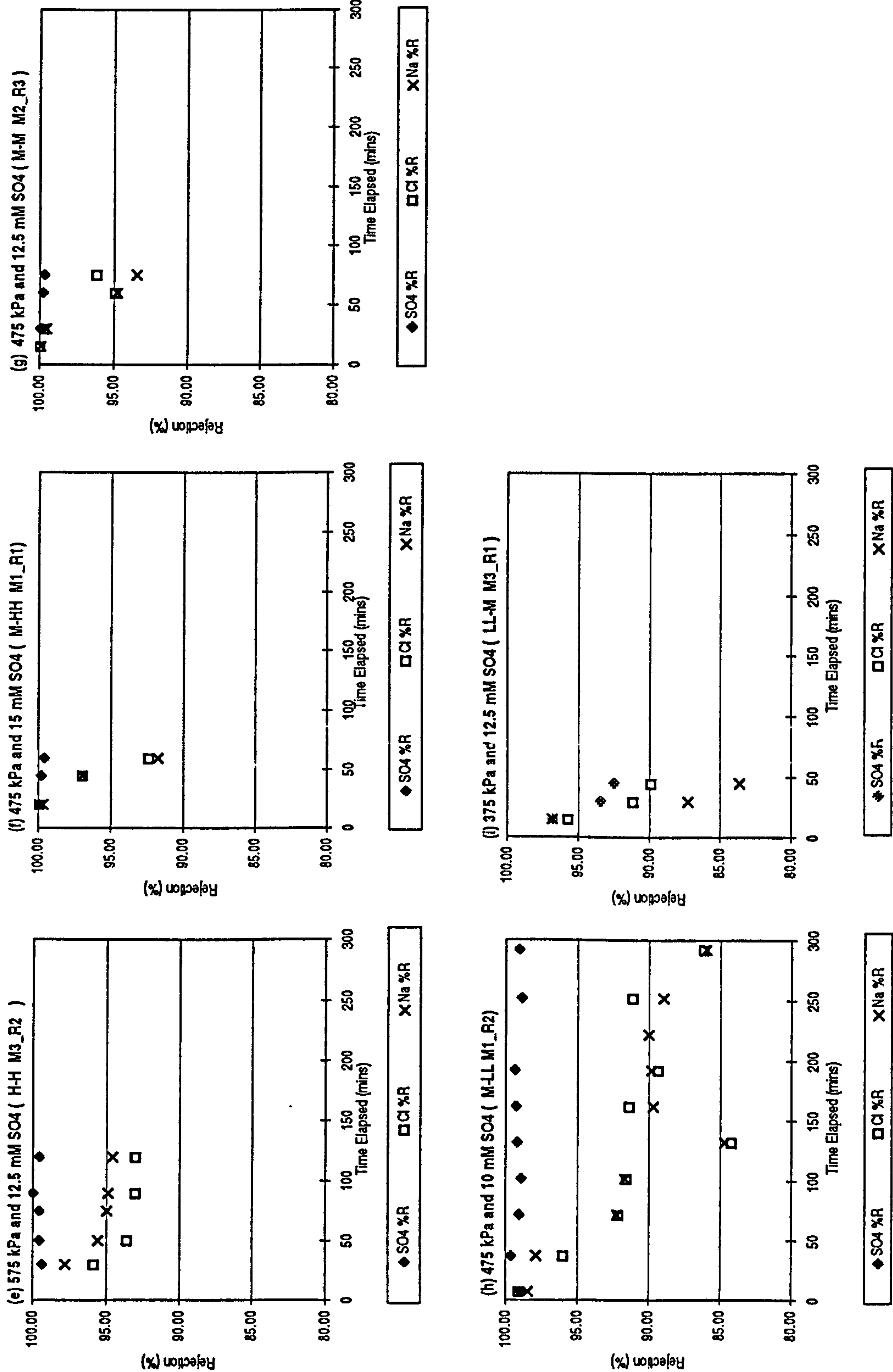
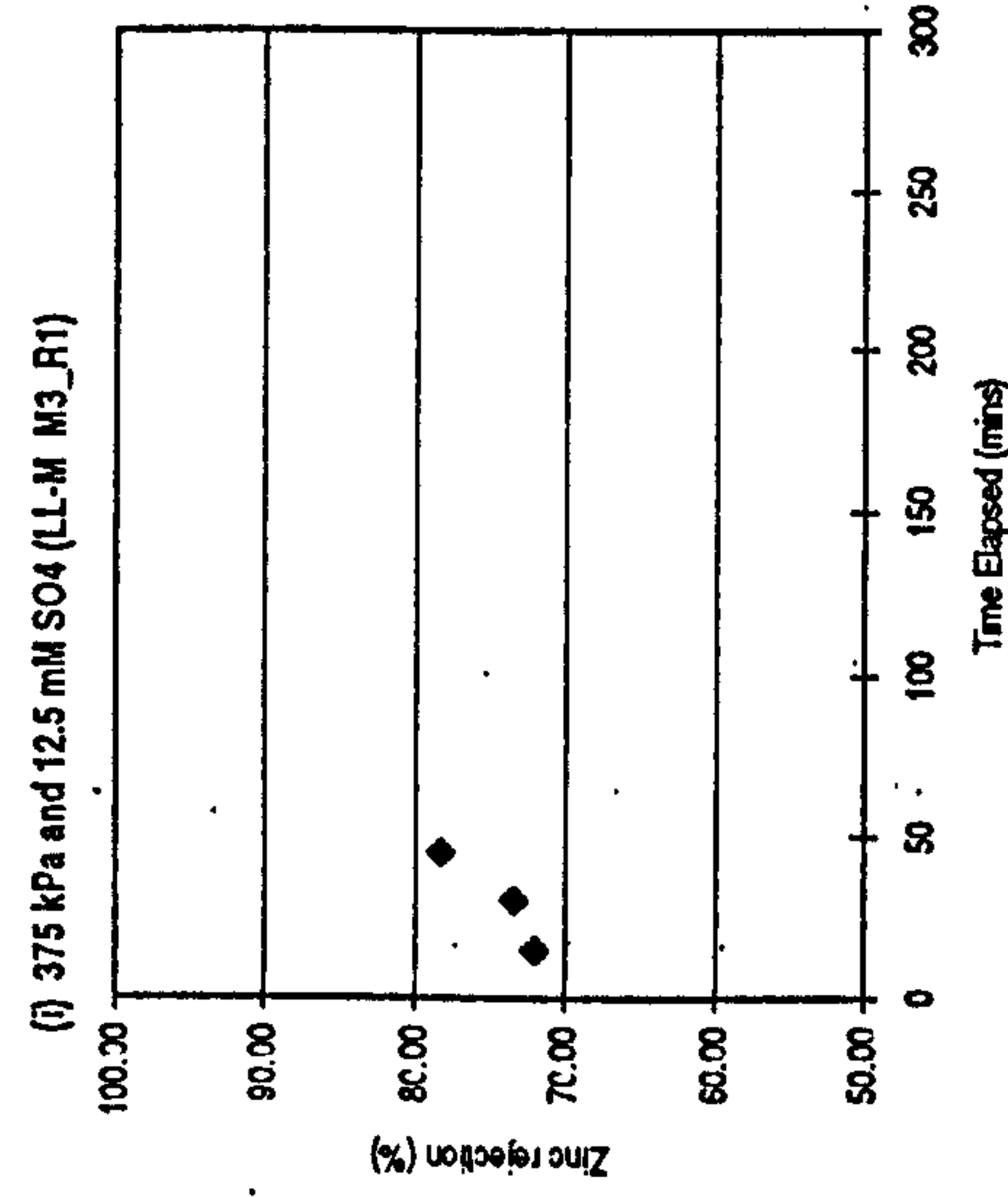
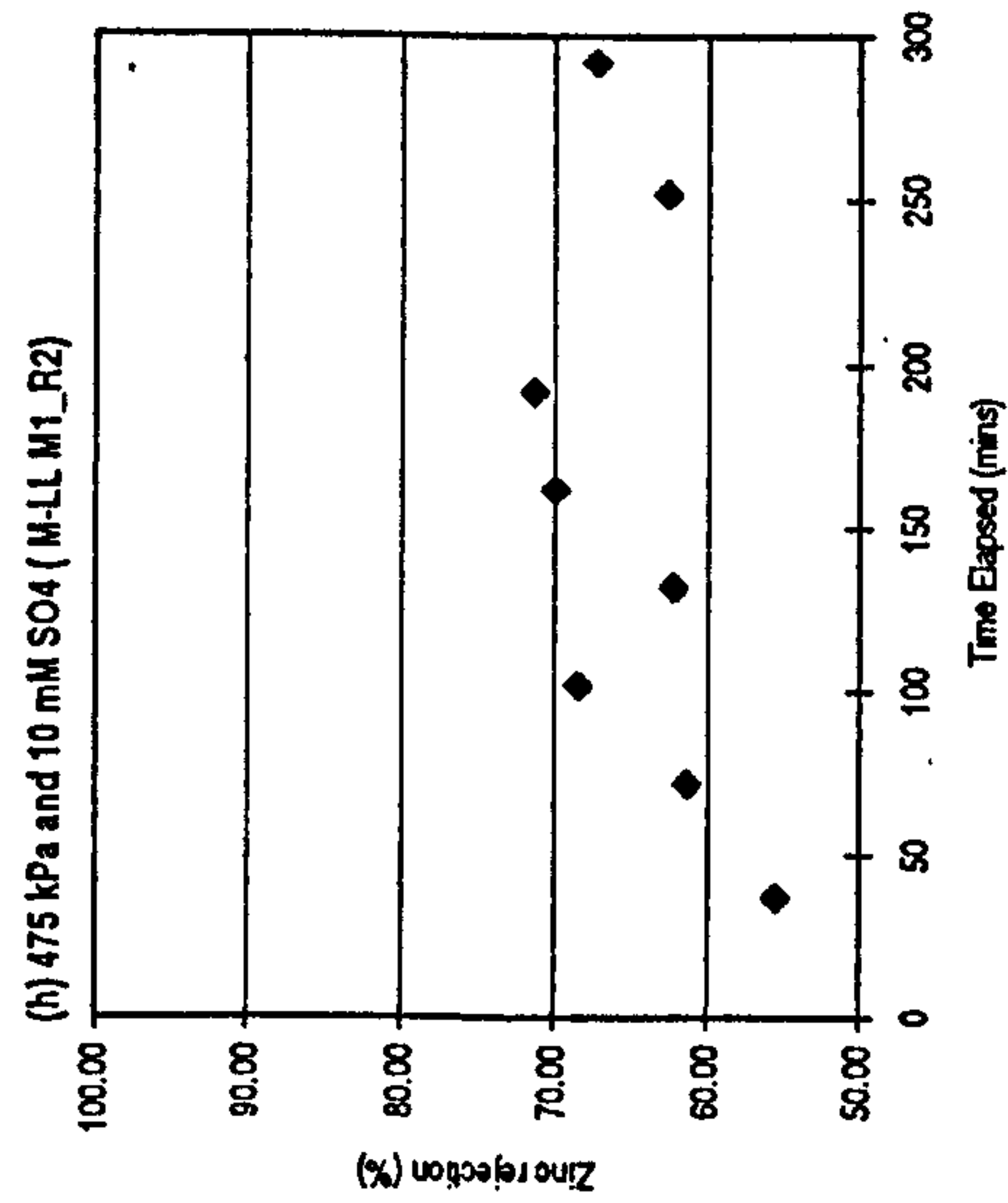
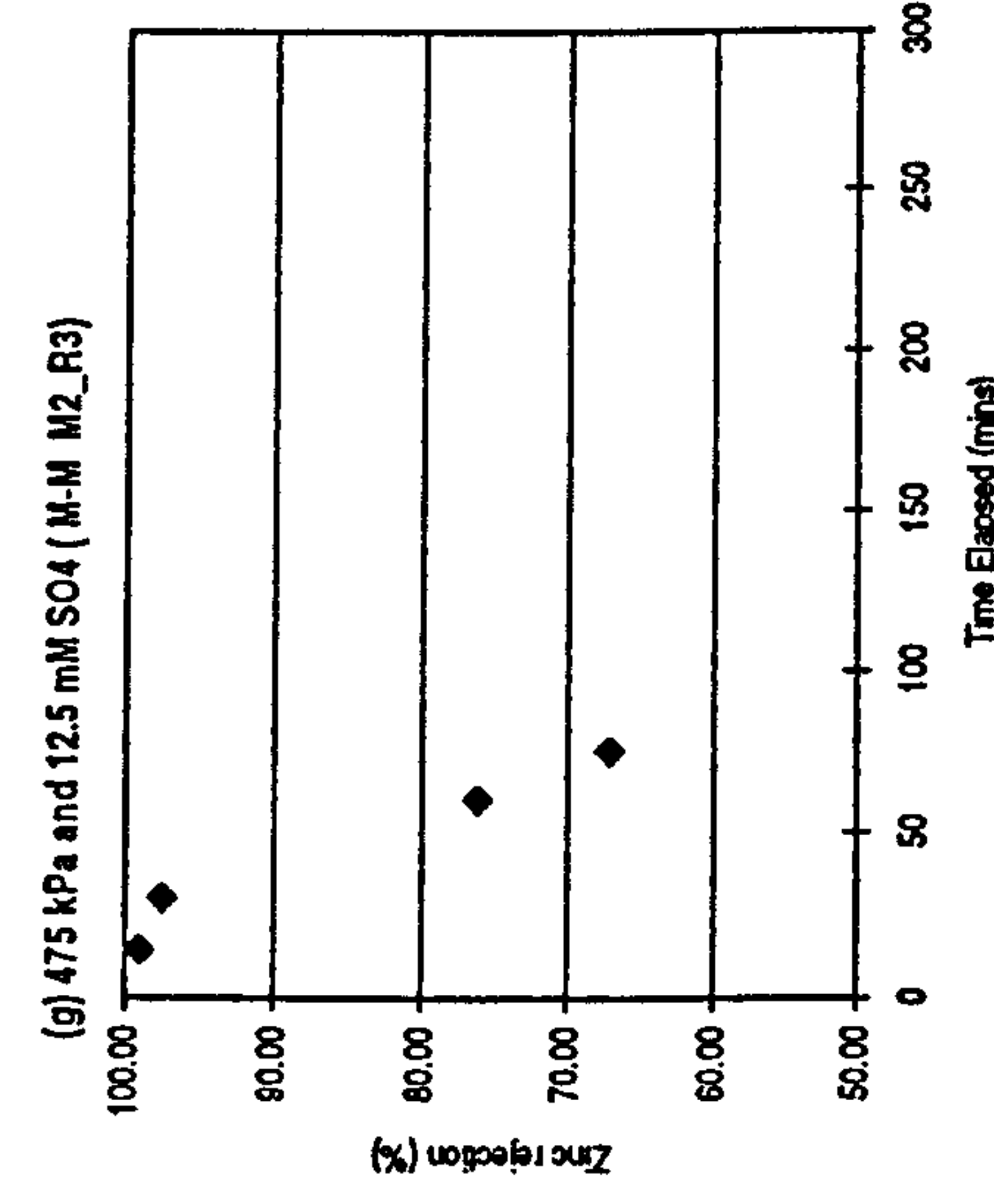
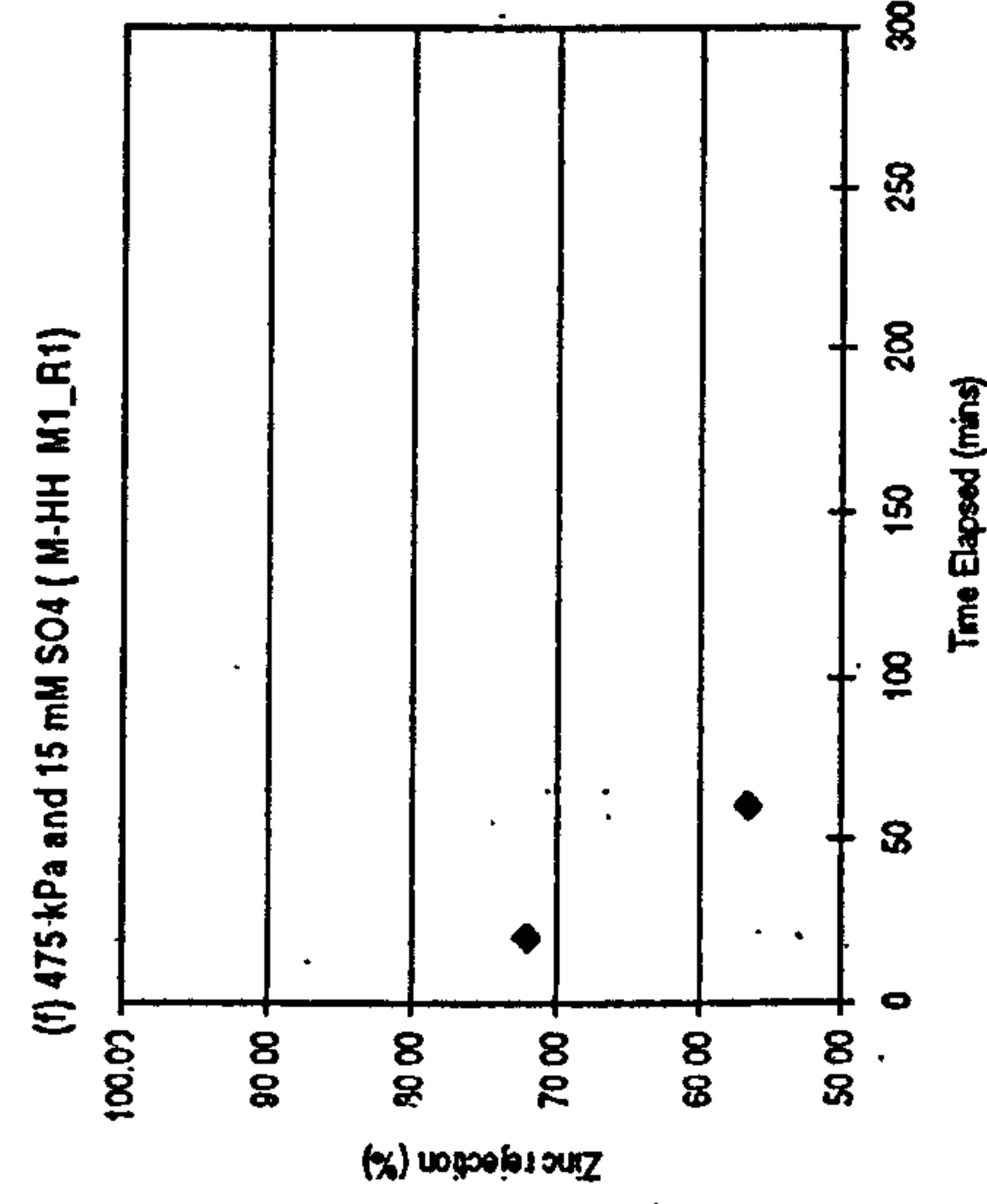
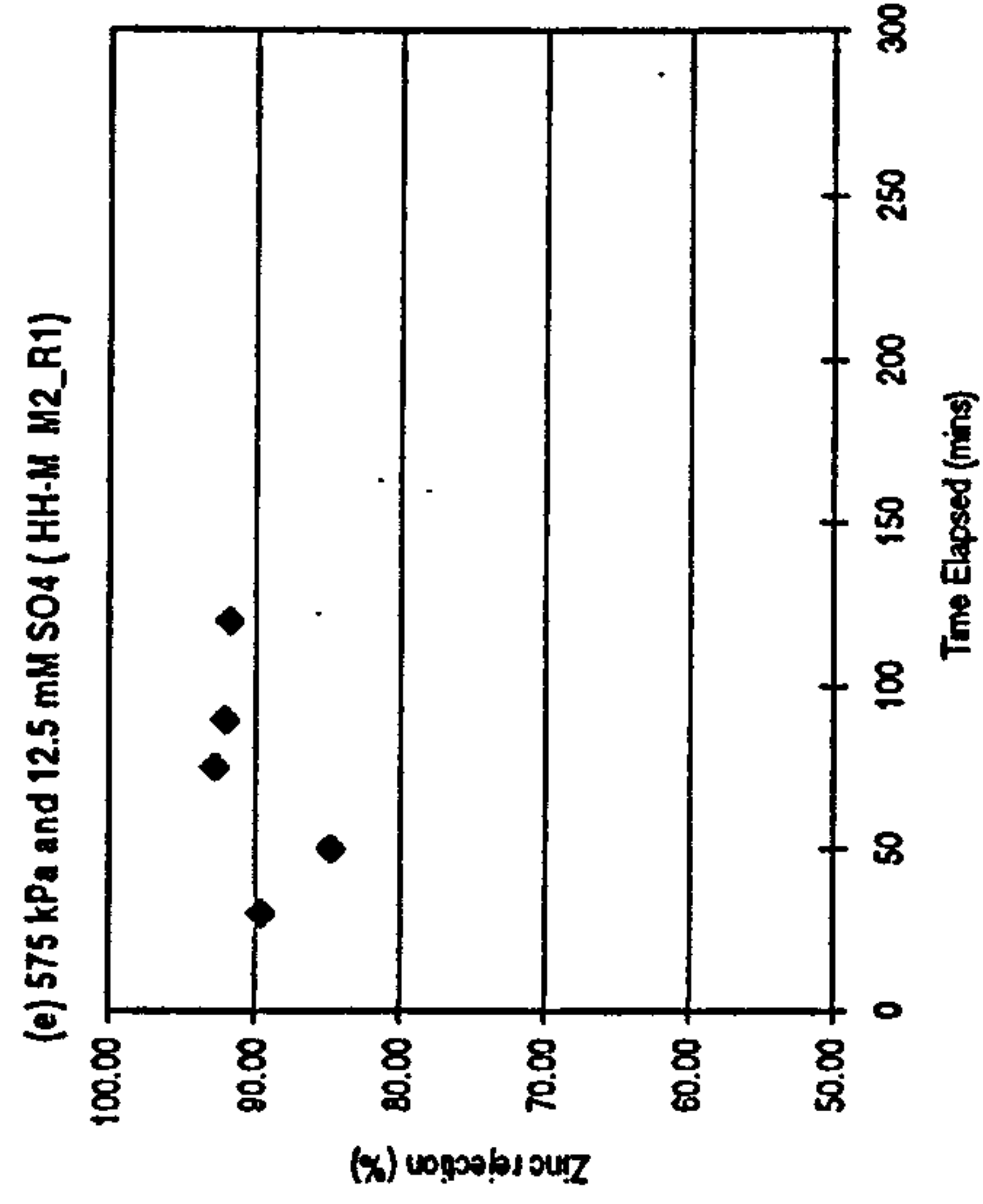


Figure 10.10 Zinc Rejection vs. Time



10.3.1 Effect of pressure

To analyse the factors that affect performance, a statistical analysis was carried out. However, the approach for analysing flux decline should consider the length of run, since it was not always the same between runs. It was decided to analyse the flux decline over a 1 hour operation. For runs which were not complete up to 1 hour, the last value was used in the analysis.

The effect of pressure on flux was in agreement with other experiments. The flux increased as the pressure increased (Bhattacharyya, 1992; Ho and Sirkar, 1992; Brandt et al., 1993). The regression equation of flux and pressure is

$$\text{Flux (l/h)} = -0.407 + 0.00272 \text{ Pressure (kPa)} \quad (10.1)$$

with an R-square value of 50.6%.

Correlation of pressure setting with rejection of some parameters showed that, as the pressure increased, the rejection of sulphate, conductivity, chloride, and sodium increased. The correlations were 0.60, 0.65, 0.62, and 0.76 respectively. Meanwhile the correlation factor for zinc rejection was 0.24. The positive correlation indicated that the rejection of parameters of interest increased as the pressure increased. This was in accordance with theory (Ho and Sirkar, 1992; Brandt et al., 1993), which furthermore explained that the rejection generally increased asymptotically (Ho and Sirkar, 1992). The correlation of rejection and pressure are illustrated in Figure 10.11 which shows that the increase in rejection performed close to an asymptotic curve. Only for Zn rejection did the curve not increase asymptotically. The reason was possibly due to the low concentration of zinc which made it difficult to measure Zn accurately in the AAS. A similar experience for low metal concentrations had been noticed by other research workers who used the same instrument. Slightly different results were obtained for separate analyses.

Table 10.1 Rejection after 1 hour

Pressure setting (kPa)	Sulphate conc. (mM)	Rejection (%)				
		SO ₄	Conductivity	Chloride	Sodium	Zinc
425 (L)	13.75 (H)	99.8	97.9	98.4	98.5	54.4
525 (H)	11.25 (L)	99.5	91.8	88.1	80.5	79.9
425 (L)	11.25 (L)	99.7	94.4	92.6	93.3	79.5
525 (H)	13.75 (H)	97.7	94.0	92.9	98.4	76.8
575 (HH)	12.5 (M)	99.6	95.0	93.4	94.9	88.0
475 (M)	15 (HH)	96.6	90.4	92.4	91.7	56.6
475 (M)	12.5 (M)	99.8	96.1	94.9	94.8	76.2
475 (M)	10 (LL)	99.2	95.8	93.4	94.1	61.3
375 (LL)	12.5 (M)	91.5	79.5	88.5	79.9	78.3

Table 10.2 Rejection after complete run (%)

Pressure setting (kPa)	Sulphate conc. (mM)	Rejection (%)				
		SO ₄	Conductivity	Chloride	Sodium	Zinc
425 (L)	13.75 (H)	99.0	91.0	90.9	88.4	82.6
525 (H)	11.25 (L)	99.3	92.7	96.3	89.9	86.3
425 (L)	11.25 (L)	99.5	92.4	89.4	90.8	76.8
525 (H)	13.75 (H)	98.5	95.0	93.5	98.4	76.8
575 (HH)	12.5 (M)	99.6	94.9	93.0	94.6	91.8
475 (M)	15 (HH)	99.6	86.9	92.4	91.7	56.6
475 (M)	12.5(M)	99.7	94.5	96.1	93.4	67.2
475 (M)	10 (LL)	99.6	93.8	89.9	89.5	53.7
375 (LL)	12.5 (M)	92.5	87.1	89.8	83.6	78.3

Further analysis of the regression equations using Minitab for the linear regression, resulted in only sodium rejection, the regression equation was statistically at 5% significance. The regression equation obtained showed 57.7% variation in sodium rejection. Others were found at a lower percentage. Considering a logarithmic equation using Excel 95, however, showed that the equations for sulphate,

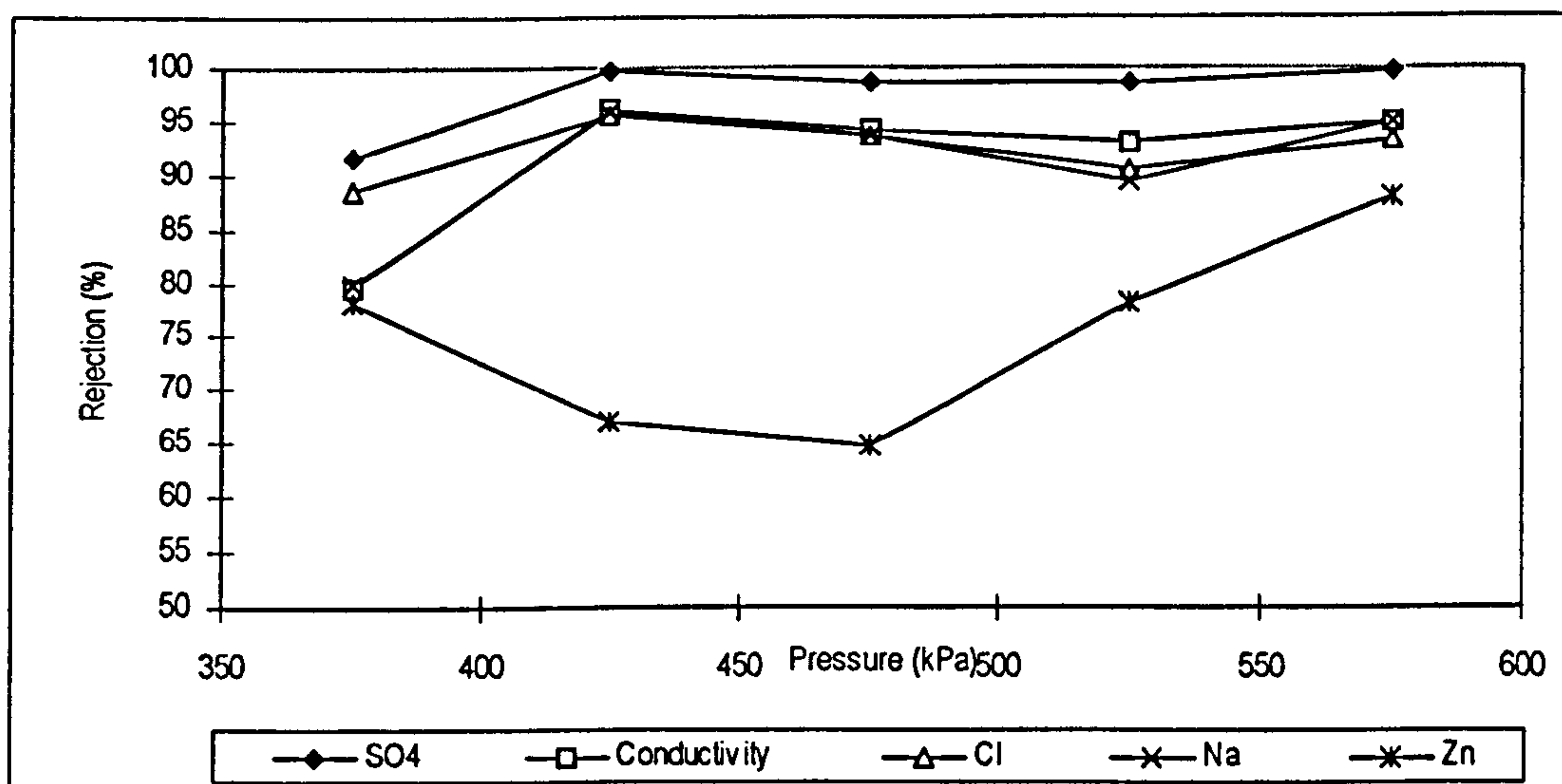


Figure 10.11 Correlation of pressure and rejection

conductivity and sodium rejection were found to have r-values of 0.72, 0.67, and 0.60 respectively.

With regard to flux decline, pressure was not correlated as its r-value was considered low (-0.348). Analysis of variance to calculate the significant effects between pressure and sulphate concentration, and any interaction between them, resulted in the rejections of sulphate, conductivity, chloride and sodium being affected by the sulphate concentration. There was no significant interaction between pressure and sulphate concentration to differentiate rejection results. The p-value for sulphate concentration was 0.087 and was considered to be significant at 10%.

10.3.2 Effect of sulphate concentration

Ho and Sirkar (1992) stated that as the solute concentration increases, the flux decreases because the osmotic pressure of the salt would be higher. In this study, correlation between sulphate concentration and flux is illustrated in Figure 10.12 showing that flux decreased as the sulphate concentration in the feed increased. Except for the lowest sulphate concentration, the curve obtained did not exactly support this theory. At 10mM sulphate concentration, the membrane used was not cleaned thoroughly. The deionised water flux after membrane cleaning could only be returned to 77% of the initial condition. Meanwhile, Siler and Bhattacharyya (1985)

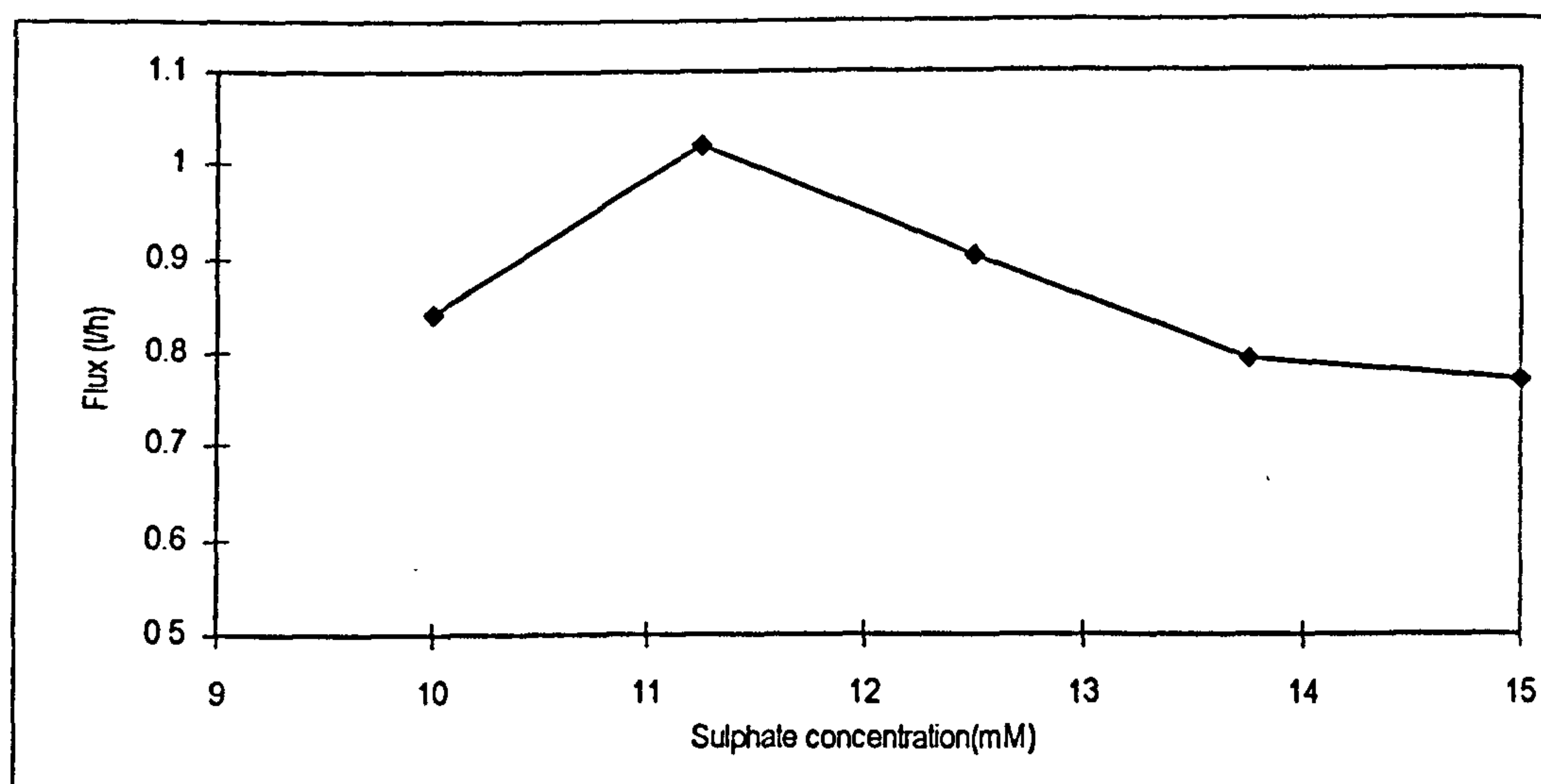


Figure 10.12 Correlation between sulphate concentration and flux

considered cleaning acceptable if it could be returned to 90% of its initial performance.

An investigation into the correlation between sulphate concentration in the feed with rejection of the parameters studied found that, from a theoretical point of view there was not the correlation expected. This was also supported by the ANOVA results of the 2^2 factorial design analysis. However, evaluation of the effect of the sulphate concentration on flux decline demonstrated that as the sulphate concentration increased, flux decline was likely to occur.

10.3.3 Fouling of the membrane

The experiment was run randomly, which implied that the configurations of pressure-sulphate concentration were run in a random sequence. This random operation maintained independence of pressure and sulphate concentration factors during the study.

Although the RO rig was equipped with pre-filter cartridges which used $50\mu\text{m}$ and $5\mu\text{m}$ polypropylene filters in series, fouling still appeared to decrease the flux. As mentioned in §10.3.2, flux decline was most affected by the sulphate concentration. The correlation factor between sulphate concentration and flux decline was high

(0.766) for 1 hour data run and 0.835 if the completed run data were used. It was a clear indication that the flux decline in the membrane was mainly caused by the sulphate concentration. It could be said that the membrane was fouled mainly by scaling of the calcium sulphate that was used in the feed solution.

Further analysis to estimate fouling of the membrane by sulphate concentration may be estimated by the regression equation:

$$\text{Flux decline (\%)} = -30.3 + 4.40 \text{ sulphate concentration (mM)} \quad (10.2)$$

The R-square of the equation was 58.7%.

10.3.4 Membrane cleaning

The study used only 3 membranes which were supplied (with compliments) of the manufacturer. The first two membranes were slightly imperfect (after confirmation by the manufacturer). Both membranes only had a flux of around 1 l/h with deionised water in the clean membrane. The third was better with a 1.55 l/h flux. This condition also affected the use of the membrane. As the membrane was used in the experiment, the fouling by calcium sulphate accumulated and cleaning the membrane for further runs should have been satisfactory.

For Membrane 1 the flux for the clean membrane was 1.2 l/h during the standardization run with sodium chloride and distilled water. Then, prior to the first run using the artificial leachate, the flux was 0.95 l/h. After run-1 and the cleaning process, the flux reduced to 0.9 l/h. Prior to the third run, the membrane only exhibited a flux of 0.82 l/h. A similar situation occurred with Membrane 2. The flux in the clean membrane was 1 l/h; then before the second run it became 0.99 l/h and before the third run the flux became 0.9 l/h. However, for Membrane 3, the flux was higher at around 1.55 l/h for the clean membrane, and before the second run was 1.18 l/h.

The data for the deionised water flux could be used to evaluate the cleaning process. It was noted that the cleaning procedure between runs for both Membranes 1 and 2 could be satisfactorily accepted since cleaning returned the membrane to 91% to 99% of the

initial condition. However, for Membrane 3 which was only used in 2 runs, the cleaning procedure failed. The second run of Membrane 3 was carried out at around 77% of the initial condition. This affected the membrane performance as shown in Figure 10.12 which resulted in a lower flux for the lowest sulphate concentration (10mM).

The study employed cleaning procedures according to the manufacturer's instructions. The methods used for membrane cleaning involved:

- i). citric acid (2%) for periods up to 6 hours at $\text{pH} = 2$ at 25°C with low initial crossflow rates (4 l/h.m^2), then at rates of up to 20 l/h.m^2 . Pressures used were up to 400 kPa,
- ii). hydrochloric acid (0.2%) for up to 6 hours at $\text{pH} = 1.6$ and 38°C , at approximately 150 kPa and a high crossflow rate of 20 l/h.m^2 , and
- iii). pumping hydrochloric acid 0.5% through the system with the membrane being left to soak overnight. This was followed by 0.5% hydrochloric acid for up to 12 hours at $\text{pH}=1.25$, 38°C , and low pressure (approximately 150 kPa) at high crossflow of 20 l/h.m^2 .

The first and second methods were used for Membranes 1 and 2, and thereafter using Method 3.

After cleaning with acid, the membrane was rinsed. At the beginning of the rinse, the flux and pH were very low. The flux was typically 0.4 l/h/m^2 , and the pH lower than 2. It was recommended to stop rinsing if the pH in the concentrate and the feed was within 1 unit pH (Amjad, 1993). In these experiments, rinsing sometimes could not be carried out in a short time. Therefore, sodium hydroxide addition was used to accelerate pH recovery. The procedure of rinsing was as follows:

- i). the cleaning solution in the tank was rinsed with tap water to remove the acid,
- ii). distilled water was added to the tank and the pH was adjusted to approximately 9 using sodium hydroxide, and
- iii). the RO unit was operated with monitoring of feed and permeate pH and permeate flux being carried out at a convenient interval until the permeate water flux was stabilized.

This rinsing process typically took several hours (3-5hours).

10.4 Pretreatment of Leachate

The first stage of the pretreatment study was to evaluate the effect of adding coagulants (FeCl_3), BaCl_2 , and the cationic polyelectrolyte Zetag 92 on a leachate sample from the site. The 2^3 factorial design was used to execute the experiment. Three factors (FeCl_3 , BaCl_2 and Zetag 92) at each of two levels (Low-High FeCl_3 concentration, with and without BaCl_2 and Zetag) were tested. Responses to pretreatment included sulphate concentration, pH, floc size, turbidity, colour, concentration of Zn, Mn, and Cu. The analysis of variance for the sulphate concentration response after coagulation is illustrated in Table 10.3.

It was calculated that the response of sulphate was significantly affected by FeCl_3 , interaction between FeCl_3 and Zetag 92, and interaction of the FeCl_3 , BaCl_2 and Zetag 92 application. Addition of BaCl_2 or Zetag 92 alone did not significantly affect the sulphate concentration. With regard to the turbidity and colour results, only FeCl_3 addition had a significant effect (the ANOVA results are not shown). None of the factors showed a significant effect on metal (Zn, Mn, and Cu) removal (the ANOVA results are not shown). However, in the evaluation of floc size, it was noted that Zetag 92 addition was important. Interaction of FeCl_3 and Zetag also significantly affected the floc size.

10.4.1 Effect of coagulant types

The effect of the coagulant types was analysed by comparing FeCl_3 , alum, and BaCl_2 addition. The sulphate in this study could only be removed at an average of 12.75%, the maximum removal being 43%. With regard to sulphate removal response, the types of coagulant did not show statistically different effects. However, the ferric chloride floc characteristic was better than that of alum (Figure 10.14). Similar results were also obtained by Sikora et al. (1989). The types of coagulant contributed to the change in pH after coagulation-flocculation. Both coagulant types and their concentration resulted in a different pH response. The pH also depended on the interaction of types and concentration of coagulant.

Ferric chloride and alum, together with polyelectrolyte addition are the most common coagulants used for the coagulation and flocculation processes in pretreatment to remove colloids before a RO unit (Bhattacharyya et al., 1992). However their effect on sulphate removal has not been widely investigated. This study revealed that sulphate could not be successfully reduced by these coagulants. Addition of BaCl₂ gave better sulphate reduction. Experiments on leachate from the same source had been previously carried out by Keogh (1997). Sulphate removal of up to 98% could be

Table 10.3 Results of ANOVA for sulphate concentration

Parameter	DF	SS	MS	F	P
Fe	1	54885	54885	8.52	0.019
Ba	1	21265	21265	3.30	0.107
Ze	1	2478	2478	0.38	0.552
Fe*B _a	1	3733	3733	0.58	0.468
Fe*Z _e	1	60910	60910	9.45	0.015
Ba*Z _e	1	19238	19238	2.99	0.122
Fe*B _a *Z _e	1	43087	43087	6.69	0.032
Error	8	51547	6443		
Total	15	257142			

Note: DF=degree of freedom, SS=sum of square, MS=Means of square, F=F ratio, and P=p-value or probability value.

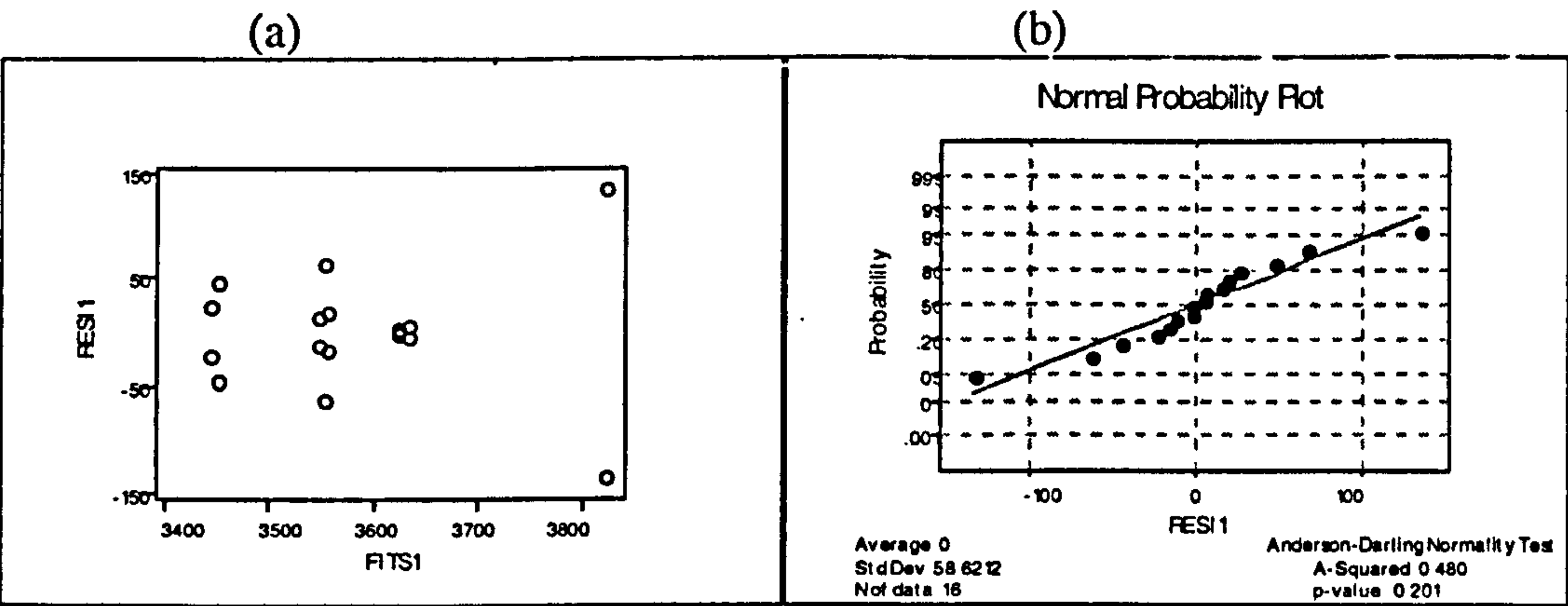


Figure 10.13 Checking of adequacy of model;
(a) Plot of residual and fitted value; (b) Normal probability of residual

achieved by using a combination of coagulant and barium chloride. The results also showed that ferric chloride addition was much better. Comparisons of removal results from both coagulants resulted in removal due to alum coagulant being statistically lower than those using ferric chloride. Other prior experiments showed that

coagulation and flocculation alone did not reduce the sulphate concentration successfully (only 12% removal) which was similar to this study. Addition of BaCl_2 made it possible to remove sulphate from the supernatants at 98%. Further acid addition also enhanced sulphate removal.

Figures 10.14 and 10.15 described the results of the coagulation-flocculation process using FeCl_3 , alum, and BaCl_2 according to dosage and polyelectrolyte (Zetag 92) addition. Although ferric chloride and alum did not greatly reduce the sulphate concentration, the flocs produced were much better than those with BaCl_2 . Moreover, BaCl_2 addition should be considered as increasing the fouling risks (Amjad, 1988; Ebrahim, 1994). Ferric chloride, if used excessively created more turbidity and colour and the floc size was no larger or no more stable. However, the use of polyelectrolyte resulted in a larger floc size if used with ferric chloride or alum, but did not affect the floc size with BaCl_2 . Care must also be taken if Zetag 92 is added, since it may increase the turbidity for all coagulants by an unacceptable amount.

The coagulant concentration affected the pH of the leachate after treatment. The greater the concentration added, the greater was the pH reduction for ferric chloride and alum. Barium chloride and Zetag 92 concentration, in contrast, did not significantly affect the pH.

A second test was carried out to further compare the use of ferric chloride and barium chloride. This demonstrated that ferric chloride was superior to barium chloride for sulphate reduction, pH, floc size and turbidity reduction. However, ferric chloride should not be added excessively since the pH could decrease significantly.

According to the factorial design results, the most significant effect on sulphate removal was when using ferric chloride, barium chloride and polyelectrolyte for the coagulation-flocculation process. Figure 10.16 shows some effects of the application of ferric chloride alone, ferric chloride and barium chloride, ferric chloride and polyelectrolyte (Zetag 92), and combining all three chemicals. As the concentration of ferric chloride increased, sulphate removal was also enhanced. However, the limitation was related to the pH of the supernatants. Ferric chloride could be added up to 200

Figure 10.14 Coagulation-Flocculation Results of FeCl_3 , Alum, and BaCl_2 addition

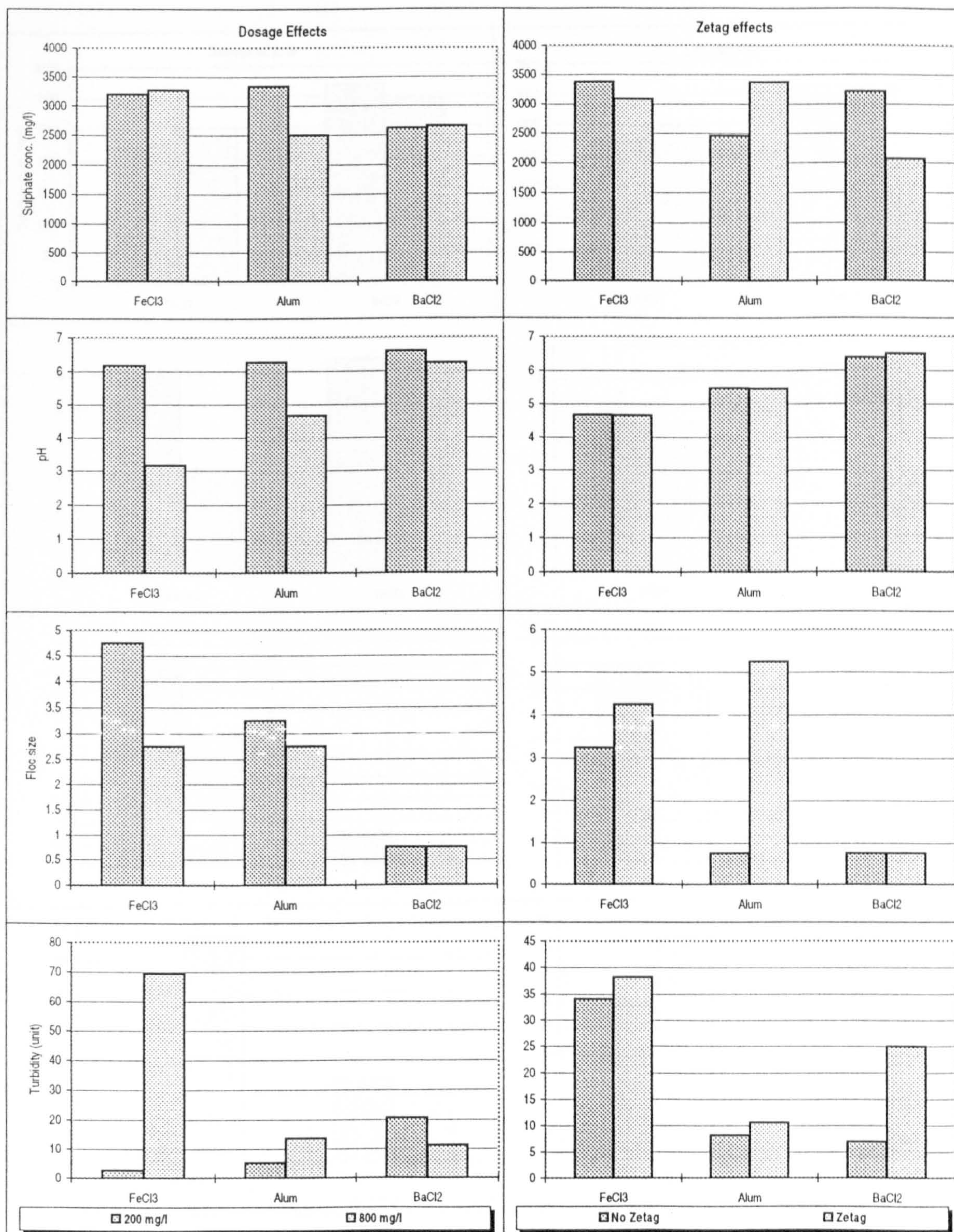


Figure 10.15 Coagulation-Flocculation Results after FeCl_3 and BaCl_2 addition

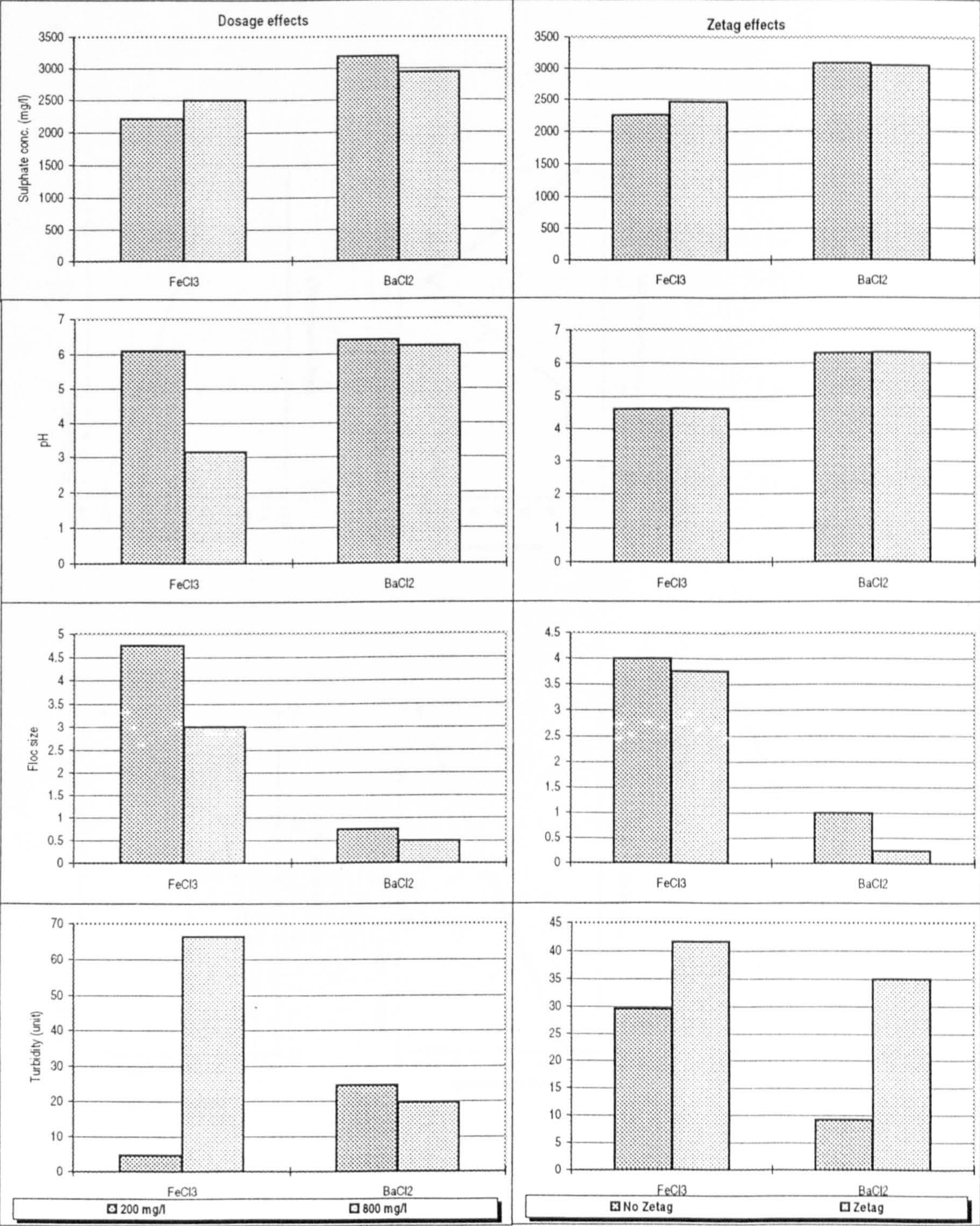
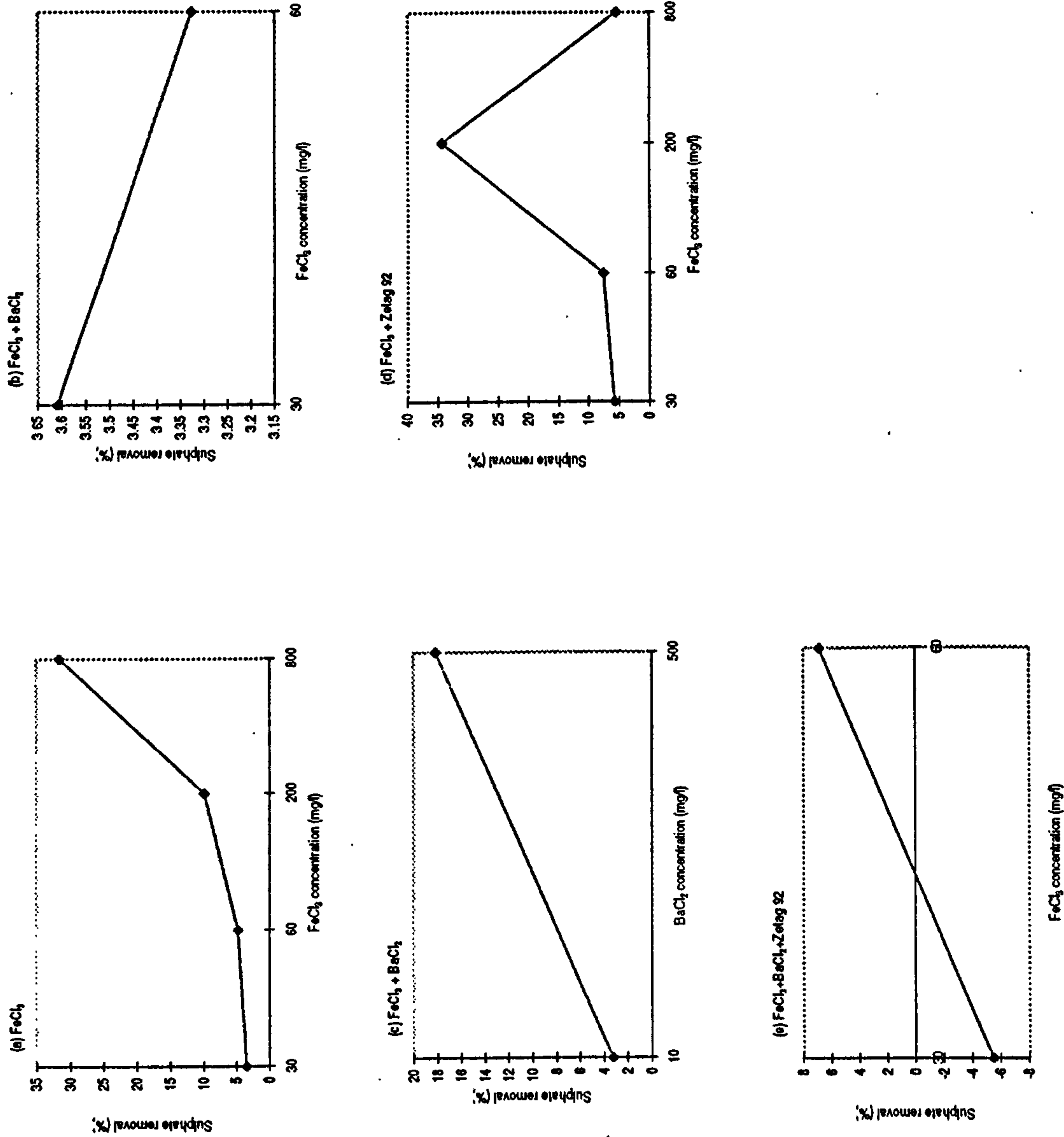


Figure 10.16 Effects of FeCl_3 , BaCl_2 and Zetag 92 to Sulphate removal



mg/l but at concentrations above this caused a decrease in pH. Barium chloride addition was also possible to be added at a higher concentration, as it increased sulphate reduction. However, the limitation was its effect on fouling and higher turbidity, and barium chloride is expensive which would significantly increase the cost of pretreatment.

10.4.2 Effect of pH

The pH of the leachate was measured under the original conditions and adjusted to almost neutral (pH = 6.6) prior to the coagulation-flocculation process. At the original pH, the process resulted in greater pH responses. From about pH 8.35 before treatment, the pH in the supernatants was in the range of 7.9-8.22. On the other hand, adjustment of pH to 6.6 before treatment showed a narrower pH range of 6.54-6.67 in the supernatants after treatment. The most important factor was that addition of either ferric chloride or alum in an excessive amount could lead to pH reduction down to 3 or 4 from the adjusted pH of 6.6.

10.4.3 Effect of barium chloride

Barium chloride, which was added to solutions with high sulphate concentration, resulted in the formation of white barium sulphate precipitates. The sulphate concentration could be successfully reduced but the precipitate should be collected and disposed of for further treatment. The supernatants of barium chloride addition were usually more cloudy than those with the coagulants. This then increased the turbidity of the supernatants. Polyelectrolyte addition did not enhance floc size from barium chloride and the turbidity level of the supernatants was greater. As the turbidity increased the colloid content may also increase which in turn increased the SDI value. This predicted the likelihood of the occurrence of scaling. In addition, the use of barium chloride would affect the chemical cost of pretreatment significantly.

10.5 Discussion

10.5.1 The use of membrane

The use of the LPROM has offered a promising treatment technology for various industrial and hazardous wastewaters (Ujang and Anderson, 1996). It significantly reduces the capital cost and energy consumption (Siler and Bhattacharyya, 1985). This has attracted its use for landfill leachate treatment. The application of RO membranes for landfill leachate treatment has been studied by many researchers (Hasbach, 1995; Linde et al., 1995; Slater et al., 1983; Krug and McDougall, 1989).

The application of RO membranes however, is prone to the troublesome problem of fouling or scaling. One of the foulants is calcium sulphate which was present in high concentrations in the leachate studied. Butt et al. (1997) found from the 'autopsy' of a membrane that scaling in a spiral wound membrane was less than that found in hollow fine fibre membranes.

This study used a spiral wound membrane configuration for the high calcium sulphate concentration in the leachate and showed that the flux decline was already present at a sulphate concentration of 11.25mM (Low concentration) after a 1-4 hour run. The sulphate concentration which did not show any fouling after more than a 4 hour run was 10mM (Low Low concentration), demonstrating that calcium sulphate caused significant membrane fouling after only a few hours operation.

10.5.2 Calcium sulphate scaling and inhibition

Amjad (1988) explained that scale on a membrane is caused by precipitation of sparingly soluble salts concentrated in the feed. He furthermore, described scale formation in three steps:

- i). supersaturation is the prerequisite for scale formation in which sulphate and calcium ions collide and form a 'cluster', the collisions becoming more active as the temperature increases,

- ii). a nucleus is created which grows larger to start nucleation, and
- iii). formation of crystals.

In real application of membrane processes, wastewater would also contain colloids or suspended matter which could initiate the nucleation process. In addition, the effect of membrane characteristics on scale is not clearly understood. A non-uniform flow could be created and result in localised areas of elevated supersaturation which could increase scale formation.

A method to reduce scaling in membranes is to use an anti-scalant which influences the rate of crystal formation (Amjad, 1985). He studied many types of anti-scalant to investigate scale inhibition and found that crystallization in the presence of anti-scalants was preceded by an induction period which depended on the anti-scalant concentration, the nature of the functional group and the molecular weight. Phosphate, for example, extends the induction period before crystallization. Polymers containing some acids are particularly effective as calcium sulphate crystal inhibitors. Amjad also concluded that the molecular weight of polyacrylate played an important role in calcium sulphate crystal inhibition.

The mechanism of scale inhibition is not fully understood. Adsorption of the inhibitors onto the crystal surface is an essential step. Amjad (1988) described two mechanisms of absorption. Inhibitor molecules absorbed fully onto the crystal to reduce the crystal growth to zero or the inhibitor molecules are absorbed onto selective faces to change the morphology of developing crystal scales.

Fountoukidis et al.(1989) proposed a model of calcium sulphate scaling on membrane surfaces. Their model was based on concentration polarization which resulted in local supersaturation and continuous deposition onto the surface of the membrane. This process reduces the permeate flux until steady state is achieved where the permeate flux is small and rejected salts are transferred into bulk solution by diffusion. The concentration of salt on the high pressure side does not exceed saturation and the deposit formation rate falls to zero. The model assumes a reduction in permeability and the rate of deposition deviates from steady state conditions. Barba et al.(1985) also proposed a model using multi-ion thermodynamics.

10.5.3 Heavy metal removal

LPROMs using a similar type of membrane have been successful in removing heavy metals such as Zn, Cd, Cu. Compared to zinc, copper removal is slightly lower. The removal of Zn and Cu depends on the zinc and copper input concentration (Ujang, 1996; Ujang and Anderson, 1996). Thomson (1996) investigated the removal of arsenic using the same membrane. He found that As was reduced by 94-97%, therefore, the application of RO for the removal of heavy metals is reliable and gives a satisfactory reduction.

10.5.4 Pretreatment of feed water

The application of LPROMs for leachate containing a high sulphate concentration is possible as sulphate was rejected at around 91-99% but the operation of the RO faced severe fouling problems. To minimise this, pretreatment of the feed is required. Coagulation-flocculation using ferric chloride, alum, and an anti-scalant have been used by Sikora et al. (1989) and H_2O_2 as an oxidant (Amokrane et.al., 1997). Chakravorty and Layson (1997) used continuous microfiltration with a 0.2 μm polypropylene membrane at 100kPa. They successfully reduced the SDI (Silt Density Index) to less than 3 and the feed was free from suspensions, bacteria, colloids, etc. Baumgarten and Syfried (1996) replaced an RO membrane by nanofiltration as the post-treatment after biological treatment of a landfill leachate. Some researchers have used hybrid membranes where the membrane operation was combined with other technologies such as ultrafiltration and biologically activated carbon (Pilbazari et al., 1996); or lower specific membrane flux in a first stage followed by a high flux membrane (Wilf, 1997). Juby et al.(1996) developed SPARRO (slurry precipitation and recycle reverse osmosis) to desalinate calcium sulphate from mine water. The system included a lower linear slurry velocity in the membrane tubes, a lower seed slurry concentration, a dual pumping arrangement to tapered membrane stack, a smaller reactor and a modified seed crystal with a blow-down system. After a 5 year-period, the system was found to be satisfactory for mine water. The energy consumption was also reduced to half of previous designs.

10.5.5 Membrane cleaning

As part of maintenance, cleaning of membranes is important. This study used citric acid and hydrochloric acid to return the membrane to at least 90% of the initial condition. Citric acid has been used to clean membranes for 30 years (Amjad, 1993). Stronger acid such as hydrochloric acid at 0.2% was recommended by Burn (1996) and then 0.5% as suggested by Cardew (1996). The study showed that Membranes 1 and 2 could be cleaned to up to 91-99% of the initial performance whereas Membrane 3 produced only 77% which was considered unacceptable (Siler and Bhattacharyya, 1989). Using 0.5% hydrochloric acid for Membrane 3 implied that descaling of the calcium sulphate precipitation had failed. The membrane specification stated that the pH range of the membrane was 2-11 for continuous operations, and 1-13 for short term exposure. The cleaning procedure could damage the membrane as it soaked overnight at low pH. Ebrahim (1994) stated that calcium sulphate and calcium carbonate could be dissolved using 2% EDTA (ethylene diamine tetra acetic acid) and citric acid which was established at pH=7 by ammonia.

10.6 Conclusions

The study showed that the application of reverse osmosis for landfill leachate treatment with a high sulphate concentration is possible in terms of its high removal. Heavy metals such as zinc and copper were also successfully removed. However, leachate with a high concentration of calcium and sulphate will create problems for the membrane as calcium sulphate deposits on the membrane. This of course will shorten the life of the membrane and consequently increase the cost of treatment. A pretreatment method may then be required to reduce scaling. Cleaning of membranes also becomes very crucial if calcium sulphate is present in the feed in high concentration.

The pretreatment study using chemicals for coagulation-flocculation did, however, not satisfactorily reduce the sulphate concentration. The coagulation-flocculation experiment could only remove a maximum of 43% of the sulphate with the

combination of ferric chloride, barium chloride and polyelectrolyte. The evaluation of the analysis revealed that the removal of sulphate could be improved by addition of barium chloride. Barium sulphate precipitates resulted in a requirement for precipitate collection and disposal. Floc settlement could be improved by the addition of polyelectrolyte. Using ferric chloride as the coagulant resulted in a better sulphate reduction, larger floc size and better settlement. However, the amount of ferric chloride should be added at the optimum concentration since it reduces the pH if excess is used. The use of a coagulation-flocculation process in pretreatment should be monitored carefully since the real leachate quality may vary with seasonal changes.

To minimise calcium sulphate fouling problems, some researchers have suggested the use of a continuous microfilter (Chakravorty and Layson, 1995), coagulation-flocculation and H_2O_2 oxidant (Amokrane et.al., 1997), a hybrid membrane (Juby et al., 1998), or the addition of anti-scalants such as formulated acrylic acid copolymer AF-1100 (Amjad, 1988), or by replacing the RO with nanofiltration (Baumgarten and Syfried, 1996).

CHAPTER 11

CONCLUSIONS AND RECOMMENDATIONS

11.1 Conclusions

The first aim of the study was to investigate the application of bioremediation for the treatment of contaminated soil having high concentrations of sulphate and heavy metals. This contaminant combination had not been previously investigated in the area of contaminated soil and the investigation was carried out through treatability tests that included the use of slurries, microbial growth studies and soil columns.

Overall, the study demonstrated clearly that slurry tests were capable of demonstrating the response of the soil under investigation to a number of environmental changes. The three methods of soil slurry test used however, showed a number of advantages and disadvantages during the investigation of specific particular soil samples. Generally, using the CO₂ evolved by respiration as an assessment of microbial activity, the slurry tests confirmed the appropriate nutrient requirements and showed the importance of the addition of a carbon source to encourage sulphate reducing metabolism. The soil required a nutrient source containing both N and P at concentrations of 20mg N/l, and 22 mg P/l. The carbon source which was shown to have a significant impact had a concentration of around 2500 µg/g soil.

The soil column showed that nutrient addition alone favoured the release of more sulphate to the leachate collected for both Soils 4 and 5. After carbon and nutrient addition on the other hand, the sulphate present in the soil was reduced microbiologically, since the microbial population increased and the sulphate concentration both in the leachate and soil matrix decreased. For the Soil 4 column, reduction of sulphate in the leachate was up to 61% whereas for Soil 5 column it was 44%. In the soil matrix, it was found that for Soil 4 column the sulphate concentration reduced from around 11600 mg/kg to 3350mg/kg. In the Soil 5 column however, the reduction of sulphate was greater after N and P nutrient

addition when the sulphate reduced from around 4600 mg/kg to 2200 mg/kg (Figure 7.36).

Throughout the study, no H₂S production was detected either in the leachate or in the gas phase. This could have been due to the conditions of soil samples that were high in pH (higher than 8) and possibly due to the column used, which was an open system and was consequently not strictly anaerobic. The reduction of sulphate possibly occurred as a result of an assimilatory sulphate reducing metabolism. This, however could not be confirmed since the study did not include the measurement of biomass in the soil sample although the metabolic process most likely occurred since it is possible to take place in aerobic or anaerobic environments.

Although the sulphate level was reduced after nutrient and carbon addition, heavy metals such as zinc, manganese, and copper were still present in high concentrations in the soil matrix. Only arsenic was removed from the soil. The strategy of heavy metal removal was then approached by leaching the metals from the soil matrix and providing further treatment in the liquid phase. This conclusion was then combined with the second investigation of the study which was aimed at investigating the application of a low pressure reverse osmosis membrane (LPROM).

A LPROM was considered the best way to treat leachate from the particular contaminated site studied. The technique offered low cost options since the energy required is much lower than for conventional reverse osmosis. Secondly, RO has been applied for treating many industrial wastewaters including landfill leachate.

The results of the study showed that the LPROM used could satisfactorily reduce the concentration of zinc (up to a maximum of around 86%) and arsenic (around 97%). It was also possible to reduce the sulphate concentration by up to 99% using the LPROM. The main problem was that of membrane fouling due to the high concentration of sulphate and calcium in the feed stream. Calcium sulphate caused scaling on the membrane surface so

that the flux was greatly decreased, thus affecting membrane performance and cleaning. The study confirmed that the flux decreased as the sulphate concentration increased.

To reduce the problem, pretreatment of the leachate was investigated using a coagulation and flocculation process with ferric chloride, barium chloride, and polyelectrolyte addition. The coagulation and flocculation process could only remove a maximum of 43% of the initial sulphate concentration. Therefore, other methods of reducing sulphate prior to a membrane unit should be investigated. The choice could include nanofiltration, continuous microfiltration or the addition of an anti-scalant into the feed-water as suggested by previous researchers.

11.2 Recommendations

Studies on bioremediation could be further developed by improving the treatability methods used for soil slurry testing and also by using more simple procedures and equipment.

The column test could also be further exploited to investigate the use of a more practical and available carbon sources and nutrients, as demonstrated by White and Gadd (1996, 1997). The results of the column tests could be extended into design and full-scale application at the field site. The bioremediation proposed by this study could reduce the time required for reducing the existing problem of sulphide gas production and leachate in the area studied.

It would also be beneficial to further investigate the microbial populations responsible for the process since this study did not include any external microbial culture addition (bioaugmentation). The organisms were already present and have been adapted to the extreme soil environment. Further experiments in a closed system are also recommended in order to investigate the sulphate reducing metabolism.

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APPENDICES

APPENDIX A

ARRANGEMENT OF EXPERIMENTAL EQUIPMENTS

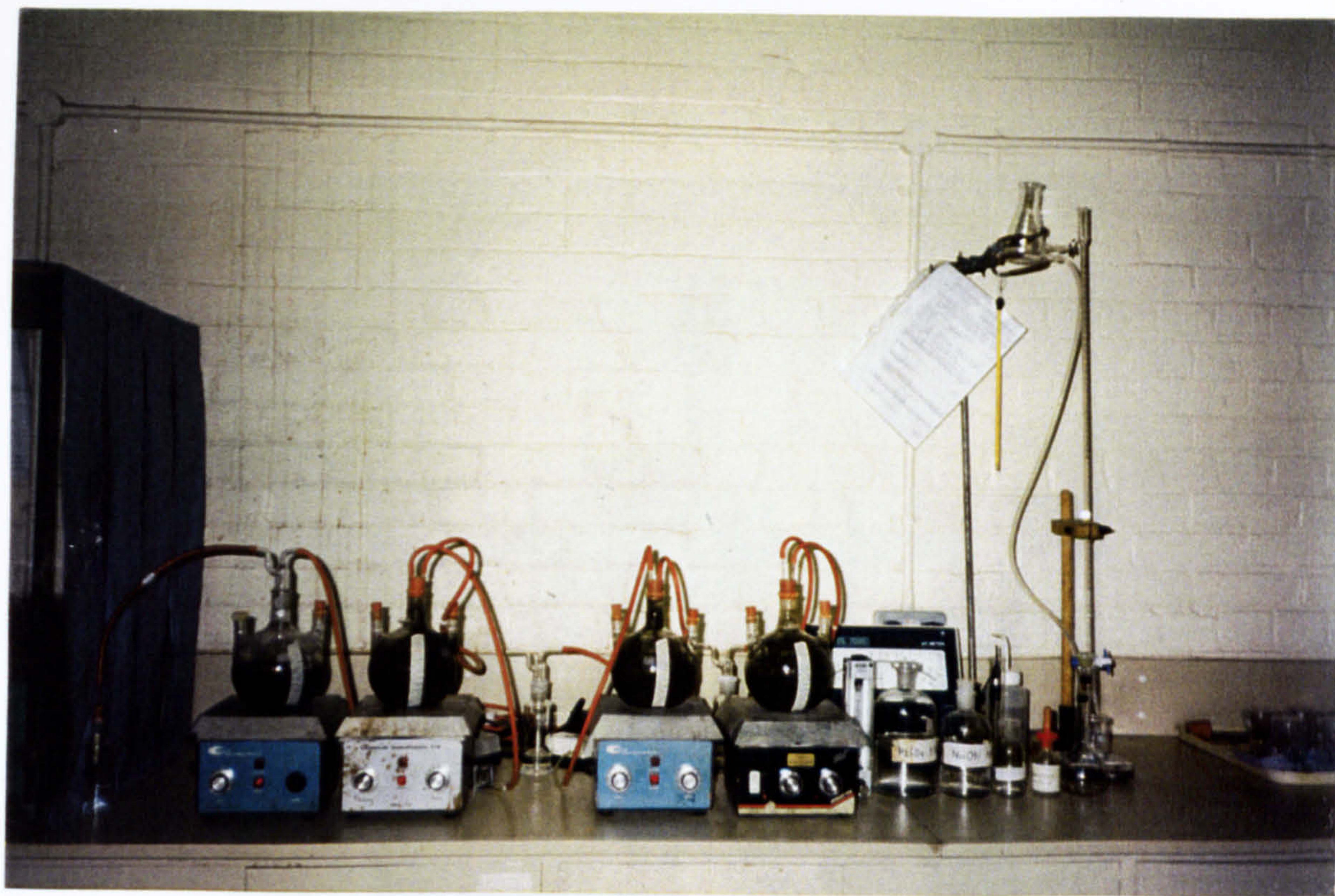


Plate 1. Equipment arrangement of soil slurry test- Method I



Plate 2. Equipment arrangement of soil slurry test- Method II

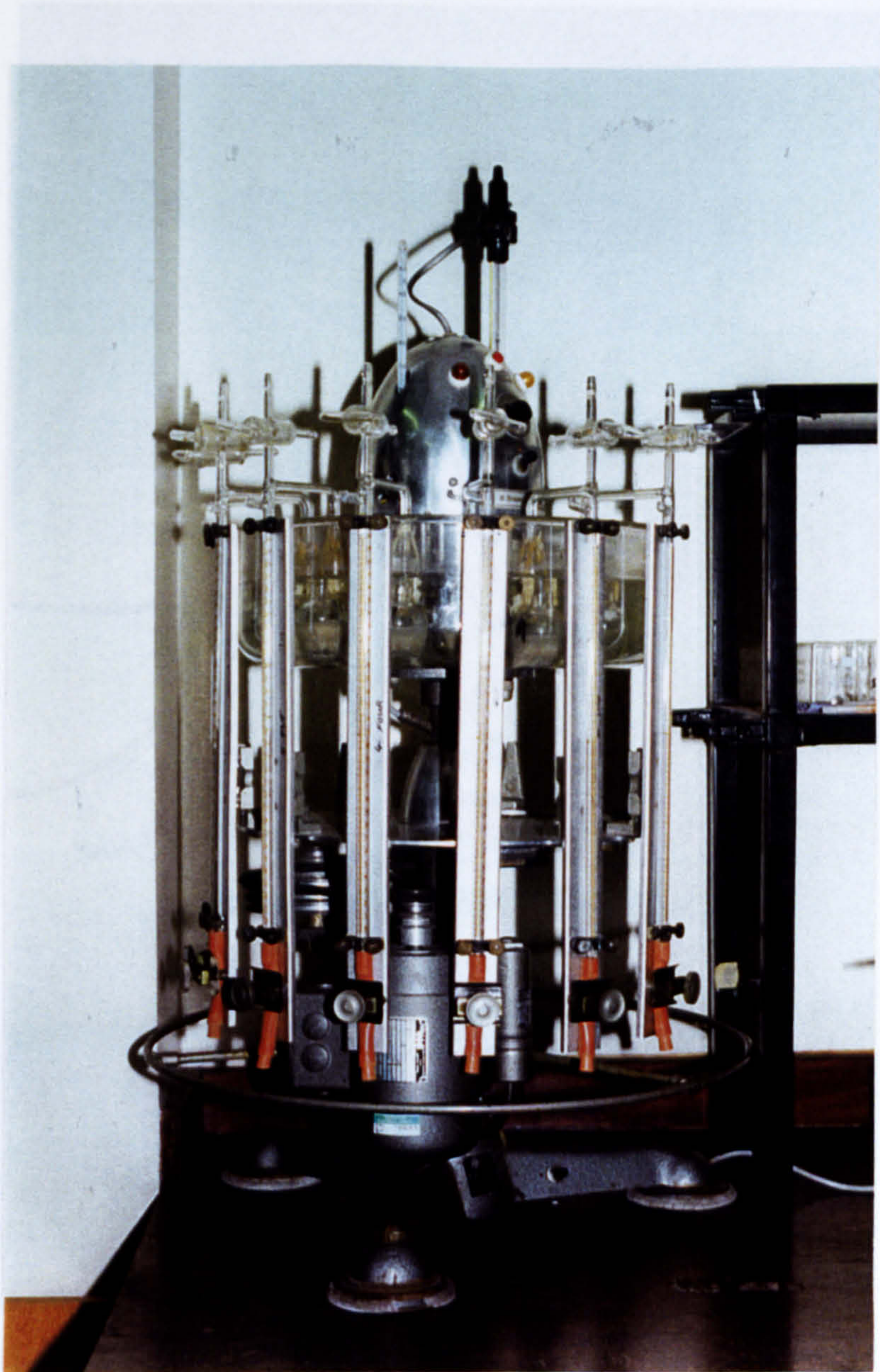


Plate 3. Equipment arrangement of soil slurry test- Method III

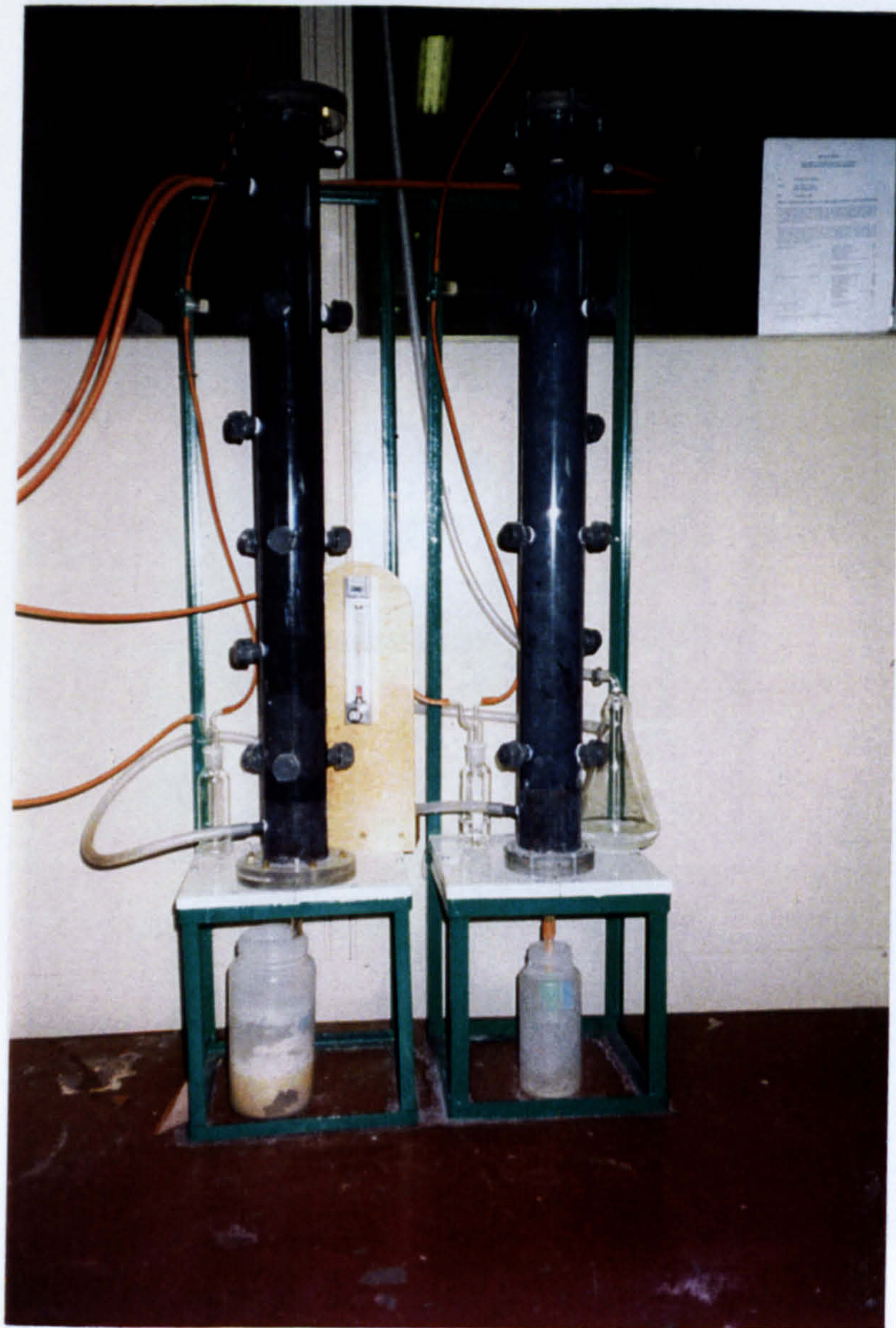


Plate 4. Equipment arrangement of soil column test

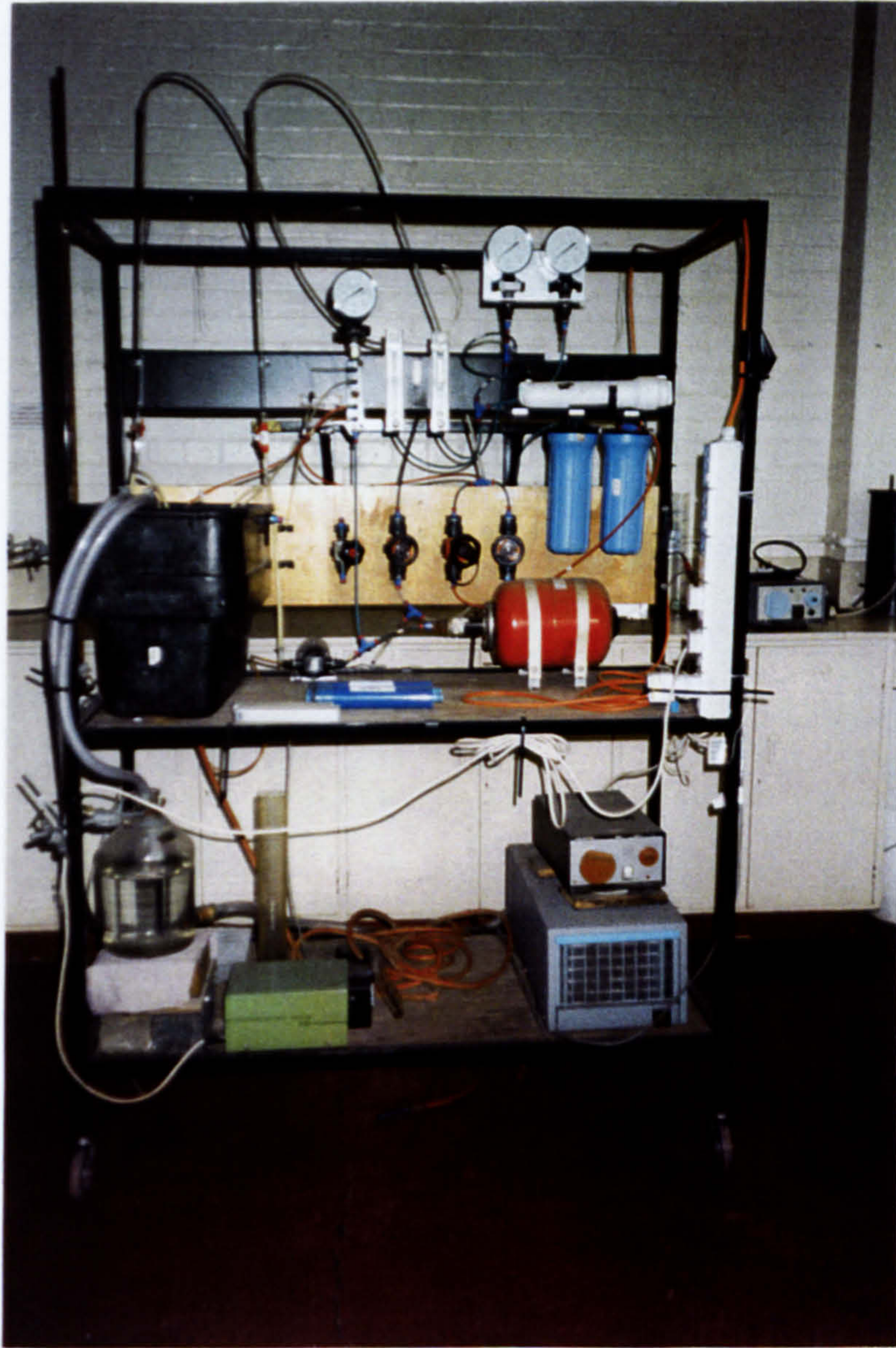


Plate 5. Equipment arrangement of Reverse Osmosis rig

APPENDIX B

CONTAMINATED SITE STUDIED

(Location of Boreholes; Leachate and Soil Qualities & Standard Criteria)

1. Location of Boreholes



2. Leachate Quality from Friar Goose Contaminated Site

Parameters	Outfalls ^{*)}	Borehole(No.3)	Trial Pit (K)
pH	6.9-8.6	7.3	7.6
SO ₄ (mg/l)	245-2230	2180	1305
SO ₂ (mg/l)	< 0.01	< 1	< 2
S ₂ (mg/l)	< 2	< 1	< 2
Cr (mg/l)	< 0.01	< 0.01	< 0.01
Cu (mg/l)	0.002-3.4	0.03	0.016
Ni (mg/l)	0.01	< 0.001	0.009
Pb (mg/l)	0.014	0.07	< 0.005
Zn (mg/l)	0.007-0.72	0.24	0.16
As (mg/l)	0.029		0.009
Ca (mg/l)	< 0.001		
NH ₄ (mg/l)	18		0.02
N (mg/l)	< 0.05	< 0.1	< 0.05
PO ₄ (mg/l)	< 0.01		< 0.01
Cd (mg/l)	0.003	< 0.001	
Hg (mg/l)	0.006	< 0.001	

Source: Appendix H, ETC Report (1995)

Note: ^{*)}The value was the maximum found from some outfalls (1,2,3,5)

3. Soil Quality from Friar Goose Contaminated Site

Parameters	Trial Pits	Boreholes
pH	4.7-13	7.6-12.6
Sol-SO ₄ (g/l)	3.26	5.05
Acid-SO ₄ (%)	16	17.59
SO ₂ (mg/kg)	58000	69600
As (mg/kg)	30	18.6
Cd (mg/kg)	19	0.9
Cr (mg/kg)	1570	155
Cu (mg/kg)	350	855
Hg (mg/kg)	26	8.5
Ni (mg/kg)	115	1160
Pb (mg/l)	2875	3270
Zn (mg/l)	345	560
Total S (mg/kg)	< 1	4450
Phenols (mg/kg)	< 1	< 0.5
CN ⁻ (mg/kg)	< 1	< 1
Total Volatile HC (mg/l)	< 20	< 20
NH ₃ (mg/kg)	880	450
NO ₃ (mg/kg)	1920	1050
PO ₄ (mg/kg)	4200	700
P (mg/kg)	< 100	< 100

Source: Appendix H, ETC Report (1995)

Note: The value was the maximum found from trial pits (C-O, Q-Z) and boreholes (No. 1-11)

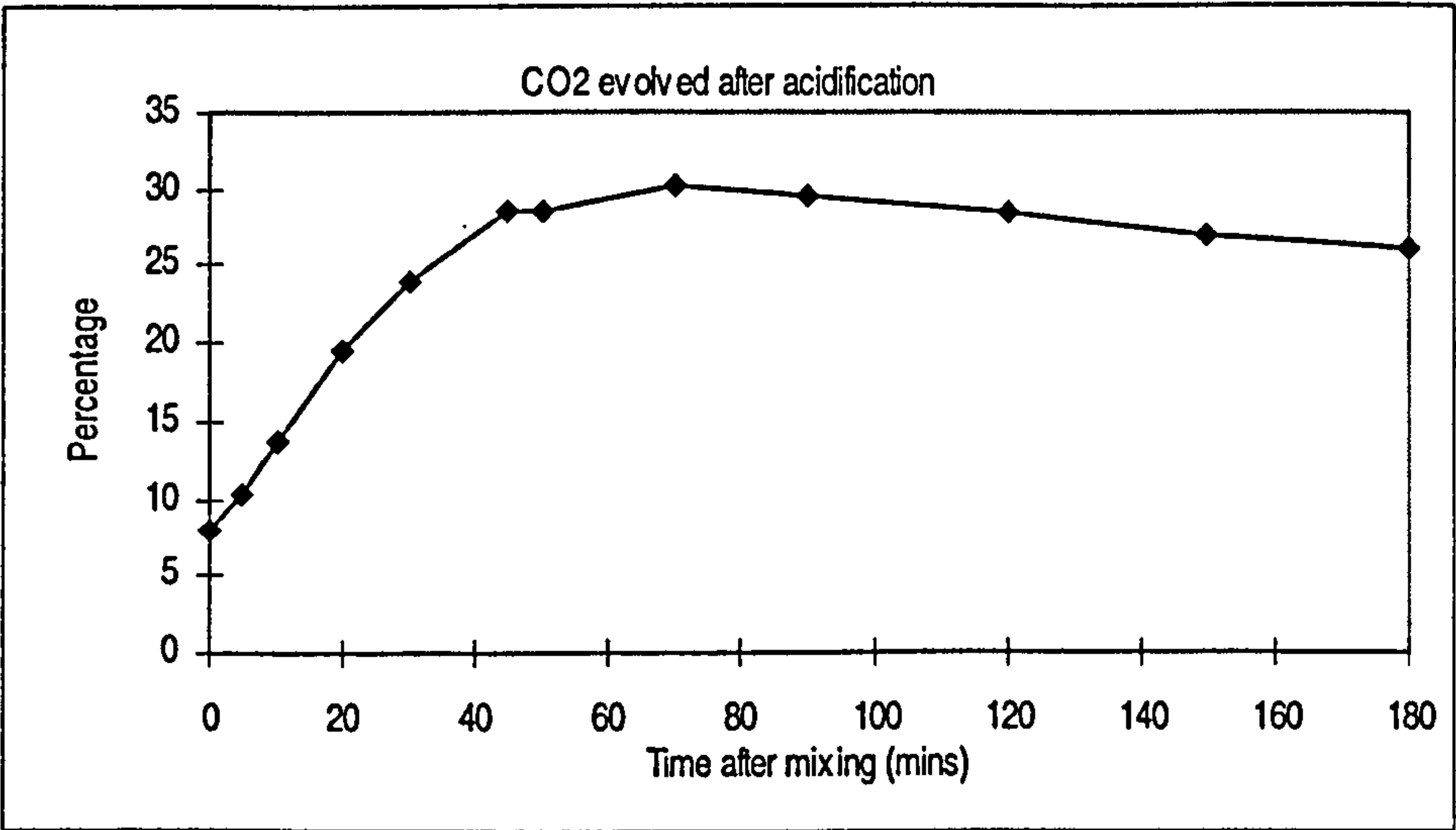
Trial pits were analysed from many depths

APPENDIX C
ACIDIFICATION OF SOIL SAMPLE

Procedure of acidification:

1. 20 gr. dry soil + 180ml deionised water were agitated for 6 hr. for soil homogenation.
2. 20ml HCl 1N was added and the mixture was left for 45min.
3. The mixture was mixed/agitated for 3 hours at room temperature.
4. Samples of gas were withdrawn to measure the CO₂ generated from the mixture and analysed in the Gas Chromatography. These was carried out several time for 3 hr.
4. The mixture was left overnight and the next day the pH was measured.
5. HCl was added until the pH was around neutral (pH=7). The mixture was agitated for 2 hours and gas sample was again measured in some intervals.
6. The mixture was left overnight and the pH was adjusted to normal again.
7. The acidification was carried out for some days and was stopped if the proportion in gas sample have relatively constant.

CO₂ evolution from acidification of Soil sample 4 during the first 3 hr. mixing:



Mixing of the mixture was carried out in 5 consecutive days for about 1-2 hours and checked later on after 2days, and one week. The procedure was completed after 12days and the pH was around 7.04 (from 11).

APPENDIX D

EXAMPLES OF STATISTICAL ANALYSES

1. Single comparative experiment

a. Two sample t-test

This example was taken from statistical analyses of Microbial Growth test procedure establishment data.

Minitab's results of the experiment: (N= Number of experiments)

	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN
No.1	27	0.6717	0.6620	0.6750	0.1374	0.0265
GFA	26	0.6677	0.6610	0.6748	0.1225	0.0240
Unshaken	35	0.7099	0.7070	0.7033	0.0806	0.0136
Shaken	18	0.5916	0.6240	0.5946	0.1680	0.0396
	MIN	MAX	Q1	Q3		
No.1	0.3150	0.9460	0.6260	0.7520		
GFA	0.3180	0.8460	0.6310	0.7500		
Unshaken	0.6030	0.9460	0.6460	0.7520		
Shaken	0.3150	0.8210	0.4760	0.6930		

i). Comparing results of Filter paper Whatmann No. 1 and GFA:

Minitab's results:

```
TWOSAMPLE T FOR No.1 VS GFA
      N   MEAN   STDEV  SE MEAN
No.1   27   0.672    0.137    0.026
GFA    26   0.668    0.123    0.024
95 PCT CI FOR MU No.1 - MU GFA: ( -0.068, 0.076)
TTEST MU No.1 = MU GFA (VS LT): T= 0.11 P=0.55 DF= 51
POOLED STDEV =    0.130
```

Conclusion: $P > 0.05 \Rightarrow$ There was no difference between Whatmann No. 1 and GFA.

ii). Comparing results between shaken and unshaken the mixture prior to absorbance measurement:

Minitab's results:

```
TWOSAMPLE T FOR Unshaken VS Shaken
      N   MEAN   STDEV  SE MEAN
Unshaken 35   0.7099    0.0806    0.014
Shaken   18   0.592    0.168    0.040
95 PCT CI FOR MU Unshaken - MU Shaken: ( 0.031, 0.205)
TTEST MU Unshaken = MU Shaken (VS GT): T= 2.83 P=0.0051 DF= 21
```

Conclusion: $P < 0.05 \Rightarrow$ Shaken and unshaken were significantly different.
Absorbance with unshaken mixture was higher than shaken mixtures.

b. Paired t-test

Data: pH of Aerobic Soil Slurry – Soil Sample 1

Date	Time	0	1	2	3	Nutrients
11-Aug	0	7.75	7.7	7.75	7.2	once supplied : 2.5ml
12-Aug	12	7.5	7.5	7.6	6.75	
12-Aug	24	7.78	7.5	7.65	6.73	
13-Aug	36	7.7	7.55	7.6	6.7	
14-Aug	60	7.72	7.37	7.48	6.55	
15-Aug	84	7.75	7.38	7.5	6.65	
16-Aug	108	7.75	7.5	7.55	6.6	
17-Aug	132	7.75	7.6	7.6	6.6	
19-Aug	180	7.75	7.6	7.52	6.55	
21-Aug	228	7.72	7.7	7.7	6.55	
23-Aug	276	7.77	7.65	7.72	6.55	
25-Aug	324	7.62	7.78	7.7	6.6	

Note: 0 = Control (aeration only)
1 = Aeration + Nutrient 1
2 = Aeration + activated sludge + Nutrient 1
3 = No aeration + activated sludge + Nutrient 1

Minitab’s results:

	N	MEAN	STDEV	SE MEAN	95.0 PERCENT C.I.
1-0	12	-0.1442	0.1541	0.0445	(-0.2421, -0.0462)
2-0	12	-0.0992	0.1220	0.0352	(-0.1767, -0.0216)
3-0	12	-1.0442	0.2013	0.0581	(-1.1721, -0.9162)
2-1	12	0.0450	0.0738	0.0213	(-0.0019, 0.0919)
3-1	12	-0.9000	0.2024	0.0584	(-1.0286, -0.7714)
3-2	12	-0.9450	0.1644	0.0475	(-1.0495, -0.8405)
	N	MEAN	STDEV	SE MEAN	T P VALUE
1-0	12	-0.1442	0.1541	0.0445	-3.24 0.0079
2-0	12	-0.0992	0.1220	0.0352	-2.82 0.017
3-0	12	-1.0442	0.2013	0.0581	-17.97 0.0000
2-1	12	0.0450	0.0738	0.0213	2.11 0.058
3-1	12	-0.9000	0.2024	0.0584	-15.40 0.0000
3-2	12	-0.9450	0.1644	0.0475	-19.91 0.0000

Conclusions: P<0.05 was significantly different i.e. pH in Treatment 1, 2 and 3 were lower than 0(control).
pH in Treatments 3 < pH in 1 ≈ pH in 2

2. Single factor experiments

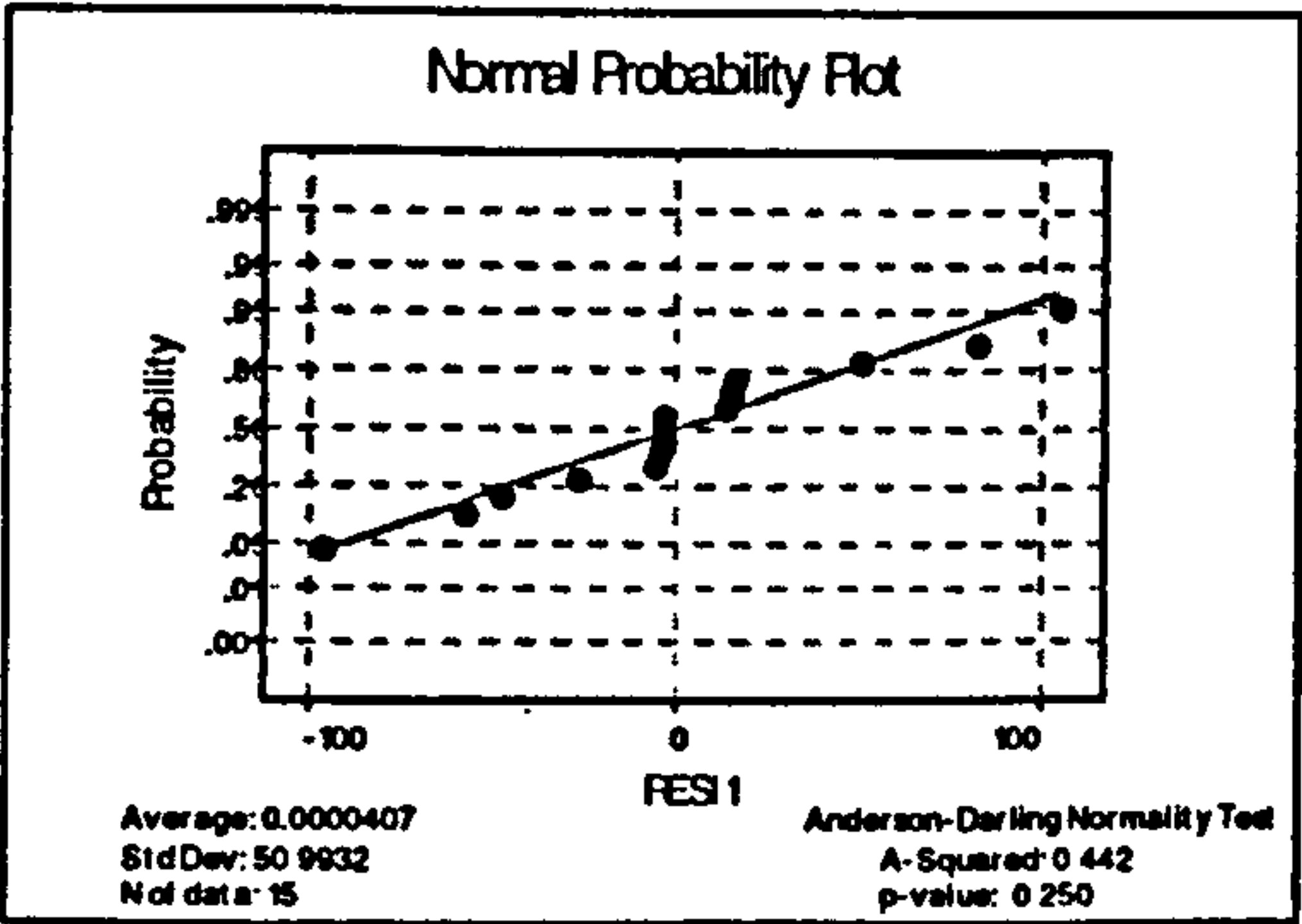
Data: Respirometer results of Soil 5 in aerobic condition for sulphate concentration.
There were 5 types of nutrients (No nutrient, N1, N2, N3 and N4) with 3 level of concentrations.

Sulphate concentration in the reaction flask after shaking (mg/l)

Level	No nutrient	Nutrient 1	Nutrient 2	Nutrient 3	Nutrient 4
Low	532.75	762.7	557.75	551.8	530.45
Medium	704.85	846.05	772.05	851.75	724.45
High	751	671.05	669.1	731.9	659.2

Minitab's results on Analysis of variance:

Source	DF	SS	MS	F	P
nutr.	4	28022	7005	1.54	0.279
level	2	93434	46717	10.27	0.006
Error	8	36404	4551		
Total	14	157860			



Conclusions: ‘Level’ of nutrient was the significant factor to differentiate the sulphate concentration.
Checking the model: data are normally distributed.

3. 2³ factorial design

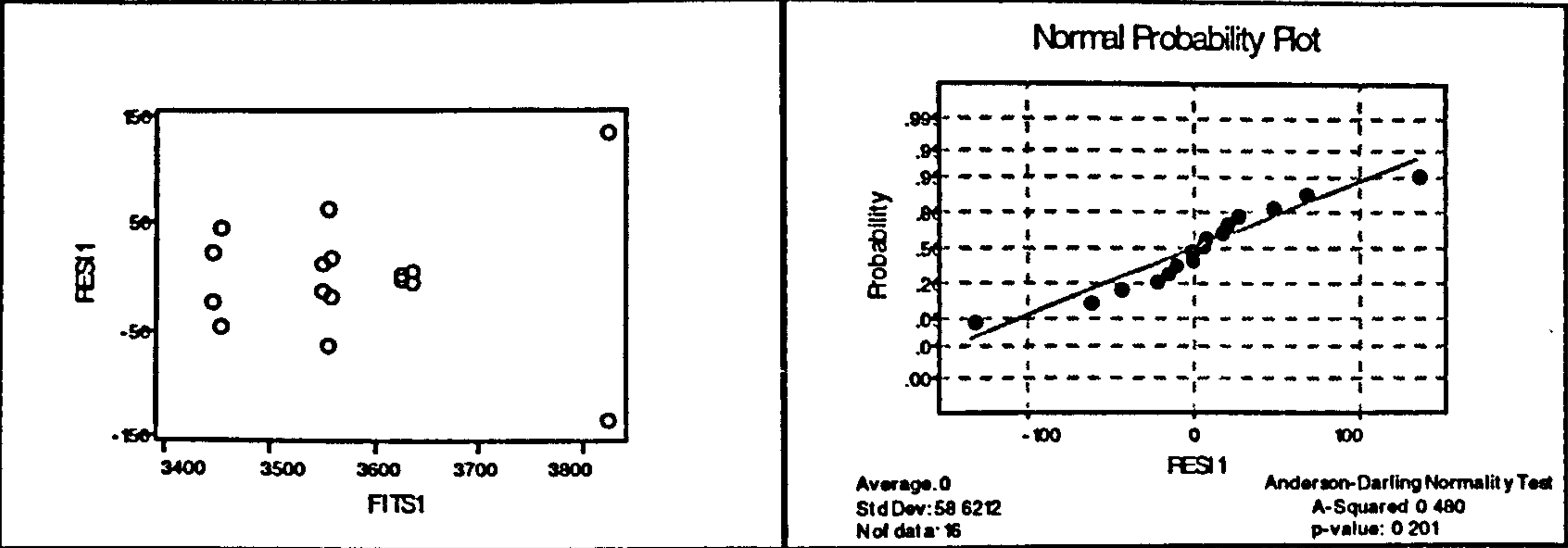
Data: Pretreatment of leachate using 3 factors (FeCl₃, BaCl₂ and Zetag 92 at two levels
(Low = FeCl₃ at 30mg/l; without BaCl₂ and without Zetag and
High = FeCl₃ at 60mg/l; BaCl₂ at 10 mg/l and Zetag 92 = 10 mg/l).
Each configuration were carried out at 2 times of experiments/replicates.

Concentration of sulphate of supernatants after coagulation-flocculation (mg/l).

Runs	A (FeCl ₃)	B (BaCl ₂)	Zetag 92	SO ₄ (mg/l)	
1	H	L	L	3536.05	3572.05
2	H	H	H	3402.15	3494.1
3	L	L	H	3560.1	3532.7
4	H	L	H	3416.9	3464.5
5	L	H	H	3687.65	3958.35
6	L	H	L	3486.45	3616.25
7	H	H	L	3634.85	3626.85
8	L	L	L	3624.15	3617.9

Minitab's results on Analysis of variance for SO₄:

Source	DF	SS	MS	F	P
Fe	1	54885	54885	8.52	0.019
Ba	1	21265	21265	3.30	0.107
Ze	1	2478	2478	0.38	0.552
Fe*Ba	1	3733	3733	0.58	0.468
Fe*Ze	1	60910	60910	9.45	0.015
Ba*Ze	1	19238	19238	2.99	0.122
Fe*Ba*Ze	1	43087	43087	6.69	0.032
Error	8	51547	6443		
Total	15	257142			



Conclusions: Fe and interaction of 'Fe and Zetag' are the significant factors to differentiate the sulphate concentrations.
Checking the model: data are normally distributed.